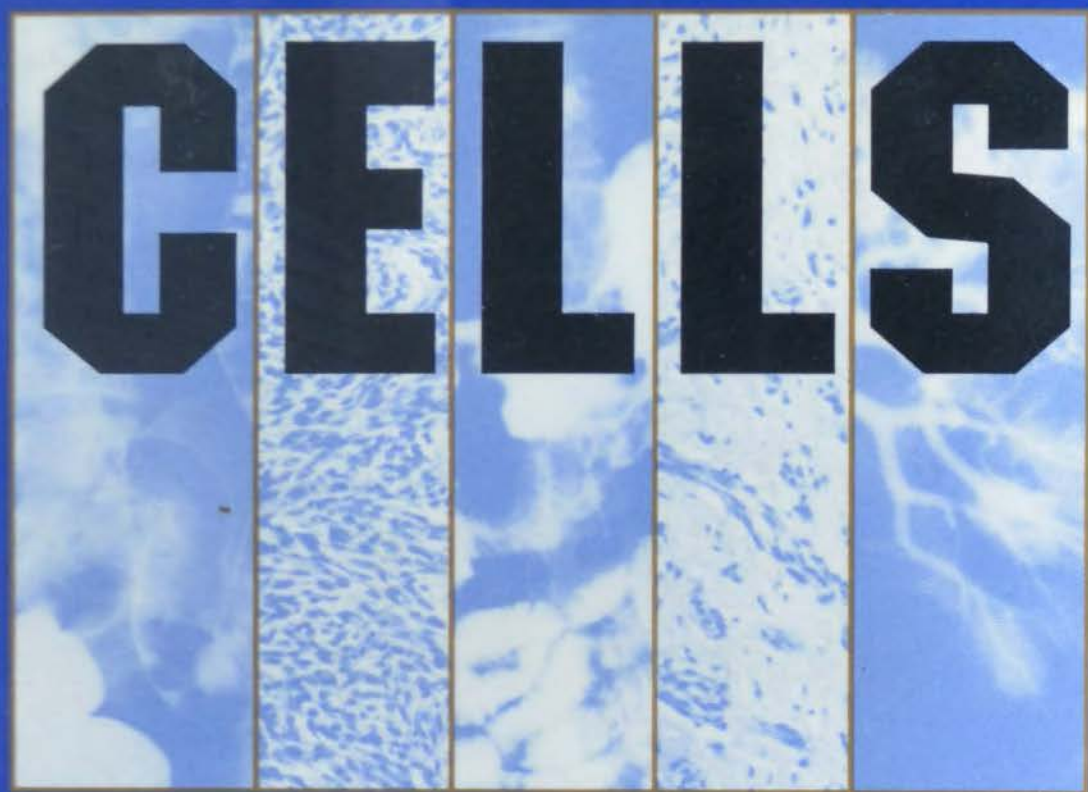


A CONSPIRACY OF



**THE BASIC
SCIENCE OF
CANCER**

R. GRANT STEEN, Ph. D.

A CONSPIRACY OF CELLS

THE BASIC SCIENCE OF CANCER

R. GRANT STEEN, Ph. D.

Directly or indirectly, cancer affects nearly everyone. The steady progress in treating cancer over the last 30 years means that more than 50% of today's cancer patients can expect to be cured and lead a normal life. Yet many of us believe that the "war on cancer" has been lost. Dr. R. Grant Steen, a veteran cancer researcher at St. Jude Children's Research Hospital, shows us that this is simply not true. By answering specific questions and providing straightforward facts, this respected and knowledgeable scientist breaks through the emotional paralysis and wall of ignorance that usually meet a diagnosis of cancer. Dr. Steen dispels the confusion and terror that surround this disease and which can often become as devastating as the disease itself. In this timely and fascinating work, Dr. Steen tackles the questions uppermost in the cancer patient's mind with clarity and compassion: What is cancer? How do you get it? How much do we know about tumor biology? How can we treat cancer successfully? And finally, what does the future hold in terms of therapy?

Drawing on over 2000 primary sources, Dr. Steen reveals the cutting edge of what today's researchers know about how cancer starts, develops, and responds to various treatments. He examines fourteen of the most common cancers, namely, leukemia, lymphoma, brain tumor, lung cancer, breast cancer, skin cancer, liver cancer, colorectal cancer, cancer of the stomach and esophagus, cancer of the head and neck, pancreatic cancer,

continued on back flap

continued from front flap

bladder and kidney cancer, gynecologic cancer, and prostate cancer. Dr. Steen firmly believes in an aggressive approach to cancer and encourages patients to take an active role in combating their disease.

The realities of cancer are painful and difficult to face. But the informed and courageous patient, armed with facts instead of fear, will be able to confront this disease with vigor and determination.



Dr. R. Grant Steen is currently studying brain cancer at the St. Jude Children's Research Hospital in Memphis, Tennessee. He is an expert in tumor physiology and tumor cell biology, and his research interest is the metabolic imaging of cancer. He employs magnetic resonance imaging (MRI) to estimate tumor perfusion, in an effort to predict tumor drug delivery. He received his doctorate from the University of California at Los Angeles, and he was further trained in research at the California Institute of Technology and the Johns Hopkins University School of Medicine. He taught at the University of Washington, where he was an Assistant Professor of Radiology and of Bioengineering, and he has written more than 25 papers which appeared in scientific journals.

A CONSPIRACY OF CELLS

THE BASIC SCIENCE OF CANCER

R. Grant Steen, Ph.D.

"Dr. Steen's book fills an important niche—that of helping individuals and their families better understand the science of cancer and the factors affecting choice of treatments and prognosis. In my experience, relief from the stress of confusion following a diagnosis of cancer can play a significant role in improving patients' attitudes, improving their relationships with their physicians, and improving the outcome of therapy."

—**Robert M. Sutherland, Ph.D.**, Vice President &
Executive Director, Life Sciences Division, SRI International,
Menlo Park, California

"This book beautifully highlights the need for research in tumor physiology—a necessary step in realizing the fruits of discovery in the molecular and cellular biology of cancer."

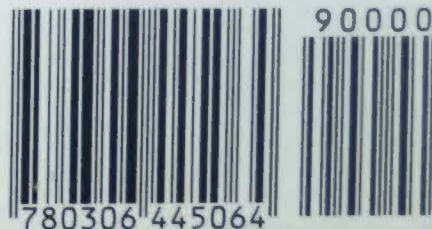
—**Rakesh K. Jain, Ph.D.**, Andrew Werk Cook Professor
of Tumor Biology, Harvard Medical School,
Boston, Massachusetts



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The treatments outlined in this volume are intended to serve only as examples. You should consult your personal physician before beginning any medical treatment regimen.

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To the Cancer Patient

At one time a diagnosis of cancer was virtually a death sentence. But during the decade of the 1980s, more than 3 million Americans were cured of cancer, and these people can expect to lead normal, productive lives. This improvement in the cancer cure rate has been hard earned: billions of dollars have been spent, hundreds of thousands of patients have been entered in clinical studies, and thousands of researchers have dedicated their lives to finding a cancer cure. But the progress against cancer will not continue unless society continues to support this vital research; only in knowledge is there hope for the future.

In generations past the relationship between physician and patient was quite different than it is today. Too often in the past the patient was seen as a passive participant in his or her own cure, with the physician perceived as wielding a godlike curative power. This situation led the patient to feel betrayed if the physician failed. Today a patient is expected to be a more active participant in therapy and a partner in the process of treating disease. Encouraging this active role in cancer therapy has led to significant improvements in the attitude and mental state of the cancer patient.

Many cancer patients find that, to take an active role in cancer therapy, they must learn more about cancer and other health issues. Several studies have suggested that patients who learn more about their disease are less depressed and tend to suffer less anxiety, anger, fatigue, and confusion. While it may be that an educated patient is simply better able to seek quality medical care, such patients may also benefit from a feeling of personal empowerment.

The psychological "coping style" used by a cancer patient apparently plays a significant role in determining disease outcome. Four coping styles have been identified: stoic acceptance, in which the patient exhibits little emotion about the diagnosis, anxiety is stifled, and the patient is passive; hopeless acceptance, in which the patient is depressed and pessimistic about disease outcome; nonacceptance, in which the patient may refuse to believe the

extent of disease and undergoes therapy merely to pacify family; and fighting acceptance, in which the patient openly expresses emotions, including negative ones, and is often combative and hostile.^{1,2} The stoic and hopeless coping styles are associated with less favorable outcomes than those experienced by patients who cope by denial or fighting. Denial leads to a better disease outcome unless the patient reaches a point at which disease progress can no longer be denied; then, as the coping mechanism fails, so does the patient. Patients with a fighting attitude live significantly longer after disease diagnosis, and this coping style is generally associated with the best long-term outcome. These patients may be alternately sad, discouraged, hostile, anxious, or combative, but they always express emotion. Although fighters are harder to deal with, for both family members and physicians, this coping style should be supported. This book is written with fighters in mind: people who want to know what they are up against while fighting their disease.

The viewpoint taken here is that a cancer patient should respond aggressively to the disease. Often an aggressive response to cancer will lead to the most favorable prognosis: early, effective local treatment, combined with adjuvant therapy for metastatic disease, is associated with the best possible prognosis. In the worst case, an aggressive response to a newly diagnosed cancer will merely confirm that the cancer is terminal. In this case an aggressive response may not buy time overall, but it will buy time between the definitive diagnosis and the eventual outcome. In almost no case will an aggressive response shorten the interval between diagnosis and outcome. An aggressive response to cancer can give patients more time to put affairs in order, more time to take leave of family and friends, more time to ensure that following generations are cared for, and more time to ready themselves for whatever follows.

Many cancer patients, especially in the last stage of their disease, become concerned with helping others who may find themselves in a similar position in the future. One practical way in which patients can help is by participating in medical research and education. This may involve entering a prospective clinical trial of a new therapy, being examined by a new diagnostic modality, or being worked up for presentation to medical students. While participation may not offer patients any real hope of a cure, the benefit to patients in the future is incalculable. Each person should remember that he or she is a beneficiary of people in the past, in similar situations, who consented to participate in medical research and education.

Acknowledgments

Anyone who has ever spent much time writing realizes the extent to which they depend on family, friends, and colleagues. Without a close-knit web of support it would not even be possible to find time to write, let alone find anything to say. My family has been called upon to be inordinately patient and forbearing, with no particular reward in return and no end in sight. My family, friends, and colleagues have provided intellectual sustenance, but, more important, many of them have also provided inspiration to persevere.

I especially want to thank my parents, Noreen and Ralph Steen, who have been very interested, very supportive, and very wonderful. My wife, Wil O'Loughlin, has borne the brunt of my daily preoccupation, with charm and good cheer, and I thank her. I also thank my children, Alena and Mariel, who provided much-needed perspective and comic relief. Probably more than anyone else, Dr. Leonard Muscatine is responsible for teaching me to think and to write; I have tried to "put the news first" for him. Dr. Jerry Glickson gave me a start in cancer research and has been a consistent friend throughout the process. Dr. James A. Nelson was a wonderful resource and helped enormously in obtaining the diagnostic images that constitute the majority of the illustrations in this book. I also thank Matthew Galvez and Dr. Linda Porter, who had faith when I didn't; Dr. George Laties and Dr. Margaret McFall-Ngai, who helped teach me to think like a scientist; Dr. Henry Brem, who provided guidance and support in the early stages of my research in cancer; Joe and Eileen Mason, who first made me aware of the need for this book; and Jeanne Fredericks, Linda Regan, Naomi Gross, and Michael Ginsberg, who helped in the practical aspects of book publication.

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CHAPTER 1

Basic Pathology of Cancer

In a sense, the cells of the human body are like the constituents of a political party. Each cell and each constituent has a particular role to play that is usually well defined. Some constituents clearly serve a function that is more vital than others, but the well-being of the whole depends on the proper functioning of all of the parts.

A political party is vulnerable to a conspiracy of its constituents. In that event, the interests of the constituents are placed ahead of those of the larger body. Often such conspiracies are of little lasting significance, and the conspirators may be isolated and killed, or they may be subsumed back into the workings of the larger body. But occasionally, a conspiracy can assume a life of its own and turn on the body from which it grew.

In much the same sense, cells of the human body may mass together and evade the normal controls imposed on them. These cells, in essence, are conspirators, although they serve no larger purpose than their own survival. Occasionally these conspirators may be isolated and killed by the immune system of the body. But if the conspirators can successfully escape the processes designed to eliminate them, their numbers can increase. If these cells continue to grow, they may form a cancer, capable of killing the body in which it grows.

While the analogy of cancer being a conspiracy of cells is graphic, to an extent it is also misleading. A political conspiracy is initiated with a specific goal in mind, whether that goal be the reformation of a political ideology or the ascendancy of a few within the power structure. But cancer cells grow with no goal other than their own survival. Cancer cells may acquire the ability to evade the growth controls imposed on normal cells through a series of accidents. Once those accidents have occurred, the cells grow simply because they can grow, not through some malevolent intention.

GROWTH DISORDERS OF CELLS

Cells of the human body are subject to several types of growth disorders, not all of which result in cancer. A fairly common growth disorder of cells is hyperplasia, in which there is an increase in the number of cells of a particular tissue or organ without formation of a tumor. Older men are frequently victims of benign prostatic hyperplasia, in which cells of the prostate form nodules that may partially obstruct the urinary tract. While this condition is certainly uncomfortable and can be fatal, it can be successfully treated by surgery. Another common type of hyperplasia affects the female breast. Hyperplasia of the breast can occur in younger women during pregnancy or in older women afflicted with fibrocystic disease. Fibrocystic disease, like prostatic hyperplasia, is easily treated with surgery. In most types of hyperplasia there is a clear expectation that the condition will reverse spontaneously or that treatment will lead to cure, without local recurrence and without progression to malignancy.

Another type of growth disorder of cells is dysplasia, which is considerably more serious than hyperplasia. This refers to variation from the norm in the size and shape of cells of a particular tissue. This variation can be accompanied by loss of normal cell orientation and by an increase in the number of cells. However, cell division in dysplastic tissue appears to be normal. A relatively common form of dysplasia is cervical dysplasia, a loss of organization and thickening of cells on the surface of the uterine cervix. This condition can be present in young women, where it may progress over a long period of time to uterine cancer, or it may spontaneously regress.

An unusual type of cell growth disorder is metaplasia, which is the abnormal transformation of a mature differentiated tissue of one kind into a differentiated tissue of another kind. An example is intestinal metaplasia, in which the lining of the stomach is transformed into glandular tissue resembling that ordinarily found in the intestine. Barrett's esophagitis is another example of metaplasia, as flattened cells that line the normal esophagus are replaced by columnar cells resembling those cells that line the stomach at the junction with the esophagus (Fig. 1). Yet another example is myeloid metaplasia, in which tissues of the spleen and liver, which ordinarily are not involved in production of blood cells, begin to produce abnormal red blood cells. Myeloid metaplasia often progresses to myelogenous leukemia, a highly malignant form of cancer.

The most serious type of growth disorder affecting cells is anaplasia, the

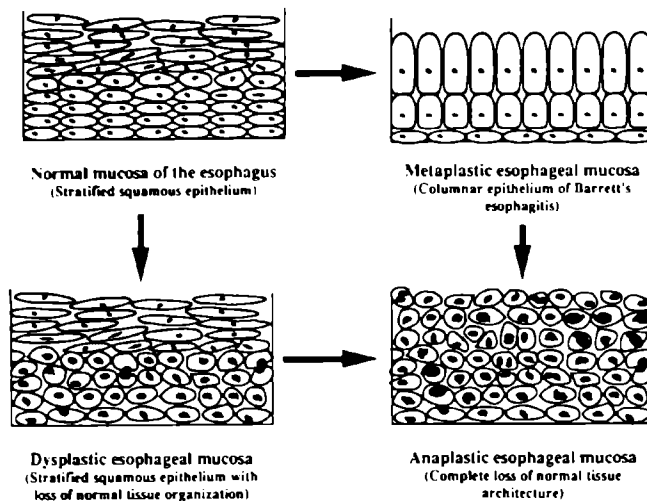


Figure 1. Highly schematic diagrams of the various types of changes that can affect a tissue. Normal esophageal mucosa (upper left) is composed of cells forming a stratified squamous epithelium. This tissue can undergo metaplastic changes (upper right) to form a columnar epithelium, which is usually found in the upper portion of the stomach. This type of metaplasia is present in patients with Barrett's esophagitis, and it can be a precursor to anaplasia. Esophageal tissue can also undergo dysplastic changes (lower left), in which there is some loss of normal tissue architecture, but there is not a great increase in the number of dividing cells and there is retention of some cells that appear to be differentiated. Dysplastic changes often precede anaplastic changes (lower right), in which there is a complete loss of normal tissue architecture. Anaplastic cells tend to have a higher rate of division (note several cells undergoing mitosis) and a higher ratio of nuclear to cytoplasmic volume.

absence of normal cellular differentiation in tissues. Anaplasia is characterized by greater pleomorphism, or variability in appearance of cells, than in dysplasia. Anaplastic cells tend to divide at a higher rate than normal cells and the cellular morphology seen during division can be quite aberrant. Anaplasia is found in most malignancies and the degree of anaplasia is often the best indicator of prognosis for a particular tumor. Benign tumors are less anaplastic than malignant tumors, so benign tumors resemble normal tissues more closely. Often, as a tumor progresses, it will become more and more anaplastic, as tumor cells achieve a more rapid rate of growth. Anaplastic cells show extensive pathological changes in their chromosomal and cellular structure compared with normal differentiated cells.

PATHOLOGICAL CHANGES OF CHROMOSOMES IN TUMORS

Pathological changes of chromosomal structure have been observed in more than 90% of human cancer, which strongly suggests that cancer results from genetic changes in cells. Chromosomes are extremely large molecules found in the nucleus of every human and animal cell. A chromosome is a combination of a very long strand of DNA and a variety of different proteins, called histones. The DNA wraps around and binds to the histones, forming a structure resembling microscopic beads on a string. The histones apparently protect DNA from damage or destruction and are also involved in packaging and replicating DNA.

Chromosomes are the repository for all of the genetic information required to build a cell. Human cells have 46 separate chromosomes, each of which is essential to cell function and each of which must be replicated when the cell divides. Since chromosomal DNA is composed of a linear array of different monomers, genetic information is actually encoded in the sequence of monomers down the length of the DNA molecule. A given chromosome usually contains enough genetic information to make thousands of different proteins. Since each protein is coded for by a single gene, chromosomes are really a series of genes, aligned end-to-end, with various bits of extraneous DNA thrown in.

Each gene of a chromosome is unique in terms of the particular order of monomers in the DNA. The order of monomers codes for and determines the order of the amino acids in proteins. Therefore, seemingly unimportant changes in the order of monomers in a gene will actually affect the structure of the protein coded for by that gene. Small changes in the DNA can, in fact, destroy the ability of a protein to function, with disastrous consequences.

Proteins are those molecules that actually do the work of a cell. They catalyze the chemical reactions involved in obtaining energy from glucose; they synthesize complex molecules from simple building blocks, so that the cell can grow and divide; they enable the cell to move or respond to stimuli; and they generally allow the cell to do the business of life. If a cell is unable to make a particular protein because of damage to the cellular DNA, that cell may die or be unable to function as a normal cell. For this reason, chromosomal aberrations are usually associated with severe pathological changes in a cell.

A number of pathological changes can often be seen in the chromosomes of various cancers. Typically, when tumor cells are viewed by microscope, they

are stained by one of several techniques, so as to improve contrast and to make cellular structures easier to see. When anaplastic cells are stained and viewed by microscope, they usually have large nuclei, with chromosomes that stain much more darkly than normal cells. This dark staining of chromosomes in anaplastic cells often makes the whole cell nucleus appear to be dark or hyperchromatic.

The various chromosomes of a cell, collectively called the chromatin, tend to be coarse and clumped together in anaplastic cells, while in normal cells the chromatin is diffuse and hard to discern. The significance of this is unknown, although it may be associated with the rapid growth rate of many tumor cells. During cell division in a normal cell, a chromosome condenses from a very long filamentous structure to a thick, stubby structure. Condensation always occurs during cell division, probably as a way to facilitate the apportionment of chromosomes equally between two daughter cells. If a cancer cell is dividing rapidly, then a significant amount of time would be saved if the chromosomes remained in the condensed state. A condensed chromosome is easier to see and may appear to be more darkly stained, simply because it is so short and thick. This accounts, in part, for why the chromatin of anaplastic cells tends to be hyperchromatic.

The chromosomal changes associated with cancer were initially difficult to recognize because chromosomes are so hard to see. The only way to really visualize chromosomes is to examine cells in which chromosomes are fully condensed, and to use specific stains on those cells. When normal condensed chromosomes are stained with certain dyes, it is possible to see characteristic banding patterns down the length of the chromosome. Darkly stained bands on the chromosome alternate with more lightly stained regions, resulting in a pattern characteristic for a particular chromosome. On the 46 chromosomes present in humans, 658 specific bands can be seen. Obviously, a scientist must be thoroughly familiar with the banding pattern of a normal cell before it is possible to recognize that a given banding pattern is unusual.

A chromosomal abnormality that is now recognized as common in malignant cells is the tendency for cancer cells to have an unusual number of chromosomes. As noted above, normal human cells contain 46 chromosomes. When a cell contains more than 46 chromosomes, that cell may be functionally abnormal. A cell that has an exact multiple of the proper number of chromosomes is termed polyploid. Polyploidy is relatively rare in cancer, because the odds are poor of randomly producing a cell with exactly three or four times too

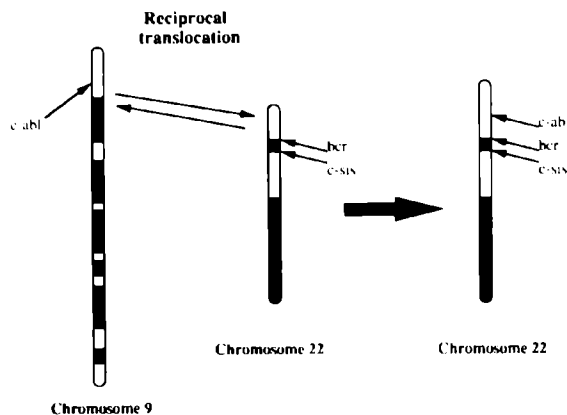
many chromosomes. Polyploidy is more frequently seen in abnormalities that arise during conception, and this kind of chromosomal aberration generally results in a spontaneous abortion.

When a cell has too many chromosomes but does not have an exact multiple of the proper number of chromosomes, that cell is aneuploid. Aneuploidy can arise during cell division if the chromosomes are not equally apportioned between daughter cells. If chromosomes fail to pair up properly during the complex choreography of cell division, then one daughter cell may wind up with too many chromosomes while the other daughter cell will have too few. Often this condition is fatal to the cell; however, if the cell survives, then all of its progeny will also be abnormal.

Aneuploidy has been seen in anaplastic cells from a fairly broad range of cancers. For example, tumor samples were analyzed from 493 patients with gastric carcinoma.³ Of those 493 patients, 37% had tumor cells with the correct number of chromosomes, while 63% had tumors that were aneuploid. Researchers have also analyzed the chromosomes of 59 patients with neuroblastoma, a tumor that ordinarily affects the adrenal gland.⁴ Of the 59 neuroblastomas, 44% had cells with the correct number of chromosomes, while 56% had aneuploid cells.

Abnormalities of specific chromosomes are seen in certain cancers. The best known example of this is the so-called “Philadelphia chromosome” (Fig. 2), which is frequently seen in patients with chronic myelogenous leukemia (CML). More than 95% of patients with CML have the Philadelphia chromosome, a very strong argument that chromosomal aberrations can cause cancer. In fact, those few CML patients who lack the Philadelphia chromosome may have a different disease. The Philadelphia chromosome results when a piece of chromosome 22 breaks off and attaches to chromosome 9, while a piece of chromosome 9 attaches to the break point on chromosome 22. This is called a reciprocal translocation. Such a reciprocal translocation may seem unimportant since, as long as neither chromosome is lost, daughter cells still have the same total genetic information. But a problem arises because the translocation brings two genes together that would ordinarily be on separate chromosomes. An unusual gene product is formed from the confluence of these two genes, and the gene product may have a role in development or persistence of CML. The Philadelphia chromosome is seen throughout the course of CML, both in remission and in relapse, and it seems to be unaffected by treatment. In later stages of CML, tumor cells may be aneuploid as well as having the Philadelphia chromosome.

Figure 2. Simplified diagram of the reciprocal translocation of genetic information from one chromosome to another that results in formation of the "Philadelphia chromosome." Only one chromosome of each chromosome pair is shown. The *c-abl* gene, from the terminal portion of chromosome 9, is broken off and exchanged with the terminal portion of chromosome 22. The break on chromosome 22 occurs at the "break-point cluster" (*bcr*) region, which is adjacent to the *c-sis* oncogene. The result is that the *c-abl* gene from chromosome 9 winds up adjacent to the *c-sis* gene of chromosome 22, so that an abnormal gene product called *c-abl/bcr* can be formed; this protein is oncogenic by an unknown mechanism.



Other cancers have been associated with other specific chromosomal changes. For example, a translocation of part of chromosome 8 to chromosome 14 is seen in Burkitt's lymphoma. Loss of one copy of chromosome 22 is found in many patients with meningioma, while an extra copy of chromosome 1 or 8 is seen in various leukemias and lymphomas. Some chromosomal abnormalities predispose individuals to develop any of several different cancers, while other abnormalities are more directly related to a specific cancer. An example of the latter is seen in patients with Wilms' tumor. Many of these patients have a particular chromosomal deletion, although only 40% of persons with that deletion develop the cancer, suggesting that a second mutational event may be necessary for induction of malignancy.

In general, leukemias and lymphomas tend to show translocations of genetic material from one chromosome to another, while solid tumors more commonly show deletions of a specific part of a chromosome. Typically, both deletions and translocations affect a limited number of sites in the chromosomes, suggesting that the affected sites may be "fragile sites," at which damage can occur more easily. The idea that chromosomes have fragile sites is consistent with the finding that certain key sites on the chromosomes are associated with induction of many different cancers.

Another type of chromosomal abnormality that can affect human cells is gene amplification, whereby portions of a chromosome undergo an increase in copy number, with the result that an increase in cellular levels of the gene product may also occur. This may be of little significance unless the amplified portion of the chromosome contains an oncogene or cancer gene. For example, amplification of an oncogene is associated with neuroblastoma, a relatively common pediatric cancer that affects the adrenal gland.⁵ An oncogene called *N-myc* is amplified in 38% of patients with primary neuroblastoma. *N-myc* amplification ranges from 3- to 300-fold, and the presence of *N-myc* amplification is correlated with rapid tumor progression. *N-myc* amplification is found predominantly in patients with an advanced form of the disease, and is predictive of poor response to therapy. The amplified *N-myc* gene appears to be associated with certain chromosomal changes present in many neuroblastomas.

When the chromosomes of a neuroblastoma patient are isolated, stained, and examined, two types of related abnormalities are often seen. Normally, stained chromosomes have a banded appearance, light bands alternating with dark bands throughout the length of the chromosome. But certain chromosomes in neuroblastoma patients have a region on one of the chromosomes with no banding. This is called a homogeneously stained region (HSR). Other patients may have little bits of chromosomal material that are not integrated into a chromosome. These bits have been called double minute chromosomes or double minutes (DMs). It is believed that DMs can integrate into otherwise normal chromosomes and that when they do so, they form HSRs (Fig. 3). This makes sense, because when a short stretch of DNA is amplified and the copies are aligned end-to-end, these copies should stain in a homogeneous manner. Since the amplified DNA probably contains the *N-myc* gene, the presence of either DMs or HSRs suggests that gene amplification has occurred. Once the *N-myc* gene is amplified, there may be an increase in the level of expression of the *N-myc* protein, or that gene may be subject to mutation.⁵

The idea that chromosomal alterations can lead to cancer is strongly supported in several ways. Three inherited human disorders (Bloom's syndrome, Fanconi's anemia, and ataxia–telangiectasia) are associated with cells that have unusually fragile chromosomes. Each of these three disorders is also associated with an increased risk that the patient will develop cancer. There is a high probability that chromosomes from these patients will suffer breakage and rearrangement when the cells are grown in culture. Another human disorder, called xeroderma pigmentosum (XP), is the result of a deficiency in the ability of cells to repair damaged DNA. Patients with XP have extreme sensitivity to

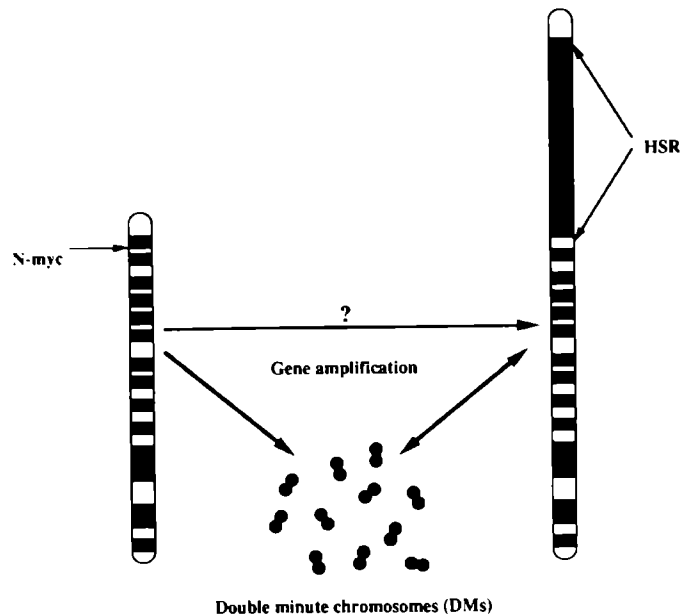


Figure 3. Hypothetical mechanism by which the human *N-myc* gene could become amplified. The amplified gene forms either double minute chromosomes (DMs), which are not integrated into the chromosome, or a homogeneously staining region (HSR), which is a linear array of amplified *N-myc* genes integrated into the chromosome. (From G. M. Brodeur, 1986, *Progress in Hematology*, vol. 14, edited by E. B. Brown. Reprinted by permission of W. B. Saunders Co.)

sunlight, and virtually all affected individuals develop some form of skin cancer. Another piece of evidence consistent with the idea that chromosomal changes lead to cancer is that many known carcinogenic agents, including radiation, tobacco smoke, and various chemicals, are chromosome-damaging agents. Thus, the known causes of chromosomal damage, whether genetic or environmental, predispose to development of cancer.

PATHOLOGICAL CHANGES OF CELL STRUCTURE IN TUMORS

Cancer is associated with pathological changes of both cellular structure and chromosomal structure. Many of the cellular changes seen in cancer are so dramatic and so obvious that even an untrained person looking at a tumor section under a microscope would recognize that the tumor is distinctly different from surrounding normal cells. Perhaps the most obvious change in

tumor cells is the dark staining of the nucleus. The chromatin of a tumor cell tends to be condensed much of the time, while normal cells show condensed chromatin only when the cells are actually dividing. Chromatin condensation means that the stain bound to the chromosome is condensed into a smaller volume. Since chromosomes are found exclusively in the cell nucleus, dark staining of chromosomes results in a dark appearance of the nucleus.

Tumor cells also appear to be more darkly stained than normal cells because the relationship between the cell nucleus and the surrounding cell cytoplasm is different in tumor cells. Using the most common cell staining technique (called hematoxylin–eosin or H&E stain), the nucleus of a cell appears dark purple, while the cell cytoplasm is pink. In normal tissue, small, darkly stained nuclei are evenly distributed over a pink background, with the nuclei representing approximately 15 to 25% of the total area visible. In tumors, the nuclei become larger, while the cytoplasm may become smaller. Darkly stained nuclei may represent more than 50% of the area viewed in a tumor section, so that the whole cell mass looks darker in a tumor (Fig. 4). The relative area of the nucleus compared with the cytoplasm is likely to undergo a progressive increase as tumor cells become more anaplastic and less well differentiated.

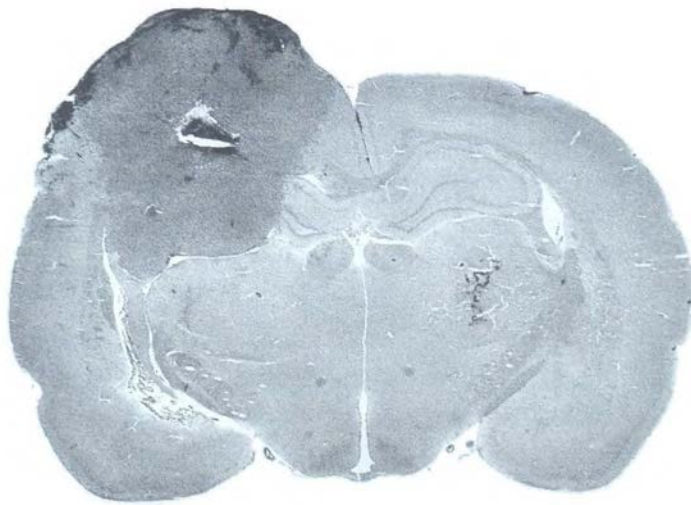


Figure 4. Rat brain (cut in cross section) with a 9L brain tumor in the upper part of the left cerebral hemisphere. The tumor is visible as a darkly stained (hyperchromatic) area with a necrotic core; a clear distinction is visible between tumor and surrounding brain.

Anaplastic tumor cells are likely to have nuclei that are highly pleomorphic, or variable in size and shape, compared with the nuclei of normal, noncancerous cells. Nuclear pleomorphism is seldom seen in benign tumors, and generally, the more pleomorphic the nucleus, the more rapidly the cells are growing. In a highly anaplastic tumor, nuclei may be so pleomorphic that they no longer resemble nuclei. The chromatin can be clumped together, or interspersed with one to many masses called nucleoli. Although a single nucleolus is found within the nucleus of a normal cell, the presence of multiple large nucleoli is associated with anaplastic cell growth. Often the number of cells dividing in a tumor is abnormally high, so there are an atypical number of nuclei caught in the division process. Anaplastic cells may also undergo a disorganized form of cell division, resulting in the formation of giant cells containing one enormous nucleus or many smaller nuclei.

Anaplastic tumor cells can also undergo pathological changes in cellular structure that do not involve the nucleus (Fig. 5). Human and animal cells contain small structures in the cell cytoplasm known as mitochondria. Mitochondria are the powerhouses of the cell, the structures within which occur

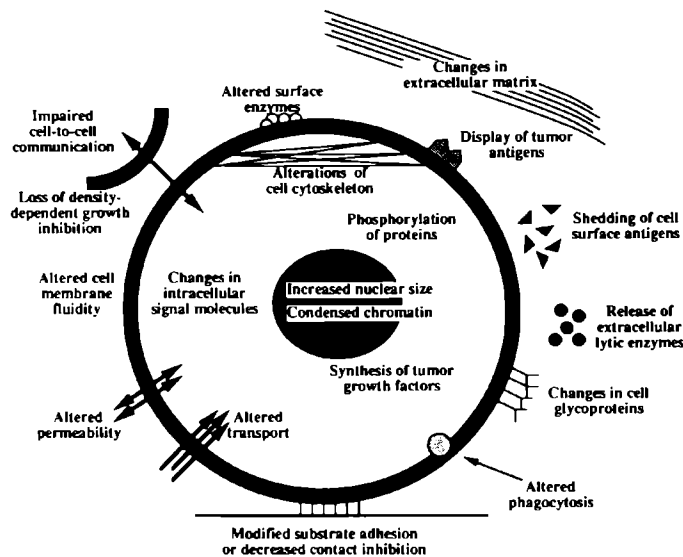


Figure 5. Diagram summarizing the cellular alterations typical of a transformed or cancerous cell. Changes can occur in the nucleus (central gray area), the cell cytoplasm (within the dark circle), or external to the cell membrane (dark circle). (From J. C. Robbins and G. L. Nicolson, 1974, *Cancer: A Comprehensive Treatise*, edited by F. F. Becker. Reprinted by permission of Plenum Press.)

many of the chemical reactions that produce cellular energy. In a given normal cell type, there tends to be a characteristic number of mitochondria per cell. In anaplastic tumor cells, the number of mitochondria per cell can be dramatically reduced. For example, in some liver tumor cells grown in culture, the number of mitochondria per cell may be reduced by as much as 80% relative to the number in a normal liver cell. This will obviously have profound effects on the biochemistry of cellular energy production.

Anaplastic tumor cells can also show abnormal alterations in the cell cytoskeleton. The cell cytoskeleton serves a support function, much as the skeleton of the human body serves a support function. In addition, just as the skeleton also enables the body to move, the cytoskeleton is involved in cellular locomotion. The cell cytoskeleton is composed of a dense fabric of microtubules and microfilaments: microtubules are rods, which are connected to one another by microfilaments, which are much like the cables of a suspension bridge. Together, the microtubules and microfilaments define the shape and orientation of the cell, enable the cell to move materials about within the cell cytoplasm, and even permit the cell to move through its environment. Tumor and normal cells do not differ in the distribution or abundance of microtubules, but recent evidence shows that the number of microfilaments is reduced in some tumor cells compared with normal cells. When a series of normal cells and tumor cells grown in culture were studied, it was found that the microfilaments in tumor cells were more sparsely distributed through the cell cytoplasm.

It has been suggested that the cell cytoskeleton forms a crucial link between the cell nucleus and the external environment of the cell, permitting the cell to respond to changing conditions in the environment. Signals from the cell periphery may be transmitted to the cell nucleus through the cytoskeleton. Since the cell cytoskeleton could thus affect the rate of DNA synthesis, it is possible that the cytoskeletal changes of tumor cells may be related to the rapid growth rate of these cells. Clearly, if the cytoskeleton is disrupted in tumor cells, this could have a major impact on the ability of the tumor cell to respond to its environment.

A final and very important way in which tumor cells differ from their normal counterparts pertains to the cell membrane. Relatively little is known about the significance of pathological changes in the cell membrane of cancer cells, yet it is known that such changes do occur. The cell membrane surrounds the cell and separates it from the external milieu, so this membrane may control the extent to which the cell responds to its environment. Changes in the cell membrane may also account for differences in the way in which tumor cells

attach to one another or respond to one another. The ability of tumor cells to grow in the absence of signals that normally stimulate cell growth may relate to unusual properties of the cell membrane. Finally, the cell membrane may control cell motility and may affect the ability of a cancer cell to spread to distant sites and form new tumors.

PATHOLOGICAL CHANGES OF TISSUES IN TUMORS

Many of the pathological changes in tissue structure associated with tumors literally grow out of the pathological changes in cellular structure. Highly anaplastic tumor cells tend to divide and produce new cells in a disorganized fashion, with a consequent loss of normal cell orientation. The result is that the architecture of a tissue can be obscured or lost, since maintenance of that characteristic architecture requires carefully controlled cell division. This is clearly seen in a tumor that afflicts the large intestine, called adenocarcinoma of the colon. This tumor can form fairly well-differentiated lesions, but it can also progress to form more poorly differentiated anaplastic lesions. The normal lining of the colon, called the mucosa, has a very specific architecture, in which a layer of muscular tissue is covered with glandular tissue that forms crypts. Crypts are tubular indentations into the wall of the colon that are lined with mucus-secreting cells having a columnar shape. These mucus-secreting cells form an even layer on the inside of the tubular crypt. In a well-differentiated adenocarcinoma this basic architecture is retained, but can be distorted. The thin layer of cells lining the crypt can become thickened, and the tubular crypts can become dilated sacs. If the adenocarcinoma cells become more anaplastic, they may completely obscure normal mucosal architecture, forming tortuous-appearing jumbles of cells that no longer secrete mucus.

When a tumor grows within a tissue or organ, tumor cells are surrounded by the normal cells characteristic of that location. As the tumor grows, it can invade the surrounding normal tissue, sending out fingers of proliferating tumor cells. The tumor can actually “capture” normal tissue by invading a region of healthy cells and growing around those cells. For example, a tumor will frequently capture a blood vessel by growing around the outside of it. This type of process produces a tumor that is a mosaic of tumor cells and normal cells. Proliferating tumor cells within the tumor mass form the tumor parenchyma, while normal cells within the mass form the stroma. Tumor stroma is partially comprised of captured blood vessels, but it can also include connec-

tive tissue and various cells of the bloodstream, including immune system cells.

Benign tumors are composed of tumor cells that may be functionally similar to their normal counterparts. As a tumor becomes more anaplastic, however, cells of the parenchyma become less well differentiated and less like normal cells in structure or function. Anaplastic tumors can have substantial variation or heterogeneity in form and function of individual tumor cells. Parenchymal cells isolated from a single tumor may vary in appearance, number of chromosomes, growth rate, response to therapy, ability to induce an immune response, potential to metastasize, and number and type of cell surface receptors for hormones or growth factors.

Some of the apparent heterogeneity within a tumor arises from heterogeneity in the growth conditions experienced by cells in the tumor mass. As the tumor grows, some cells find themselves farther and farther from blood vessels that perfuse the tumor, so that they may not receive all of the nutrients necessary for survival. These isolated cells gradually starve, first ceasing to grow, then beginning to die, and become necrotic. Occasionally, a large region of the tumor will become necrotic and actually liquefy, forming a fluid-filled space called a cyst. But cystic spaces within a tumor can be scattered among viable tumor cells that continue to proliferate.

Cells, whether normal or tumorous, grow within an extracellular matrix of material that acts as a scaffolding. This extracellular matrix may provide support and protection for the cells, and it may also be involved in maintaining normal cell differentiation and cell-to-cell communication. Extracellular matrix is difficult or impossible to see in most microscopic views of tissue, so the concept that all cells grow in such a matrix may be unfamiliar. The matrix is composed of proteins woven together into a dense meshwork, within which cells are nestled.

Significant differences between normal cells and tumor cells can usually be seen with regard to the extracellular matrix material. Tumor cells often overproduce matrix materials, which can lead to an accumulation of excess matrix around cells. Alternatively, when tumor cells invade normal tissues, they may secrete proteins that disaggregate or digest normal extracellular matrix. In any case, the result is that tumor cells can be surrounded by matrix quite unlike that surrounding normal cells. Extracellular matrix may play an important role in regulating gene expression in cells, so abnormal matrix may contribute to the abnormal growth pattern of tumor cells.

TUMOR NOMENCLATURE

Tumor nomenclature, or the system of naming a particular tumor, is important because the name is a kind of code word by which physicians summarize a wealth of information about the tumor. The tumor name therefore conveys a great deal of information to the oncologist. Tumors are named using a system that is based on several features of tumor biology:

1. Anatomic site of the primary tumor (e.g., lung, colon, breast)
2. Classification of the tissue of origin (e.g., epithelium, connective tissue)
3. Determination of the degree of cell anaplasia (i.e., is the tumor benign or malignant?)
4. Extent of tumor progression (i.e., degree of invasion and metastasis)

A difficulty that can be encountered in the clinic is determining whether a given tumor is a primary tumor or a tumor metastatic from a primary site located elsewhere in the body. For example, suppose that a small tumor is noted in the lung during a routine chest X ray. If this tumor is a primary bronchial carcinoid, the tumor will probably follow a benign course, and the 5-year survival rate for such a patient is about 95%. But if the lesion is a tumor metastatic from an undiagnosed or occult tumor at another site, patient prognosis can be radically different. For example, certain patients have what is called an extragonadal presentation of testicular cancer, with normal-appearing testes but an advanced germinal cell cancer in part of the lung. These patients have a low cure rate, a high relapse rate, and a 5-year survival rate of only about 20%. Fortunately, it would be relatively easy to determine whether a given lung lesion was a bronchial carcinoid or a germinal cell cancer, using some of the diagnostic tests available to the physician.

Determination of the primary site of a tumor is critically important because this will dictate several features of the clinical course of the cancer. Primary site in large part determines: the likelihood of metastatic spread, and also the organs most vulnerable to metastasis; the effect of the tumor on normal organ function; and the treatment options available to the patient. Determination of the primary site of a tumor is not always easy, especially when dealing with a highly anaplastic tumor that no longer resembles the tissue of origin.

Even when the primary site of a tumor is known, the diagnosis of tumor type and the determination of patient prognosis is not a simple matter. To return

to our example of a small tumor observed in the lung during a routine chest X ray, let us suppose that this lesion is known to be a primary tumor. As we noted, the 5-year survival rate for a patient with a bronchial carcinoid is 95%. But it can be difficult to distinguish between a bronchial carcinoid and small-cell lung carcinoma (SCLC), since both tumors are derived from the same normal tissue. Patients with SCLC may have a good initial response to therapy, but the tumor typically recurs in a form highly resistant to therapy. About 70% of patients with SCLC have distant metastases at the time of diagnosis, making a complete cure nearly impossible to obtain. The life expectancy of a patient newly diagnosed with SCLC is often less than a year, and the 5-year survival rate is only about 12%. Even if the tumor is localized, with no invasion of adjacent structures and no evidence of metastatic spread, the 5-year survival rate is only 40%.

Classification of the cell type from which the tumor originated is very important with respect to patient prognosis. This is because the tissue of origin can often determine the biology of a tumor. This is not always true, because both benign carcinoids and highly malignant SCLC are probably derived from cancerous transformation of the same precursor cell. But, generally speaking, the tissue of origin can reveal significant clues to patient prognosis.

Most benign tumors are composed of cells that closely resemble cells in the tissue of origin. These tumors are often named by attaching the suffix “-oma” to the cell type from which the tumor arose. Thus, a benign tumor arising in fibrous tissue and composed of cells called fibrocytes would be called a fibroma, while a benign tumor arising in cartilaginous tissue would be called a chondroma. Benign tumors of epithelial cells, those cells forming the surfaces of organs or tissues, do not follow this pattern because there are simply not enough names to denote the many different types of epithelial cells. Accordingly, benign epithelial tumors are classified on the basis of their microscopic appearance or cell of origin. Benign epithelial tumors that have a glandular pattern of cells, or that are derived from glandular tissue but that may lack the glandular pattern, are called adenomas. Benign epithelial tumors that produce a raised, warty appearance on a surface are called papillomas or polyps. Benign tumors that form large cystic masses, as in the ovary, may be called cystomas or cystadenomas. Occasionally, a malignant tumor will be called an “-oma,” but that tumor will then usually be designated as malignant. A good example of this is malignant melanoma, a very aggressive and malignant skin cancer that should properly be called a melanocarcinoma.

The nomenclature of malignant tumors generally follows the same pattern

as seen in benign tumors, with a further layer of complexity. Malignant tumors of the epithelial cell layer are called carcinomas, so a cancer of the cells lining the lungs would be a carcinoma. A carcinoma of the lung would be further classified on the basis of the microscopic appearance of the cells. If these tumor cells form a structure resembling a gland, the tumor would be called an adenocarcinoma. If the tumor is composed of flattened, scaly epithelial cells, it would be called a squamous cell carcinoma. Sometimes a carcinoma retains enough of the differentiated features of the tissue of origin that the tumor is named after that tissue. Thus, a well-differentiated malignant tumor in the kidney would be called a renal cell adenocarcinoma. But sometimes a tumor is so anaplastic that the precise tissue of origin cannot be identified, in which case the tumor might be called a poorly differentiated carcinoma.

Malignant tumors can also arise in cells other than those covering a tissue surface. These tumors are commonly referred to as sarcomas. A malignant tumor of fibrous tissue origin would thus be called a fibrosarcoma, while a malignant tumor of cartilage would be a chondrosarcoma. Most malignant tumors are composed of one proliferating cell type, but occasionally a tumor will be composed of more than one cell type. If all of the cells of the tumor are derived from one broad class of cells (e.g., all epithelial cells), the tumor may be called a mixed tumor. If the tumor is comprised of a broad range of different cell types, it may be called a teratoma.

The degree of anaplasia of a tumor is critically important in determining whether a tumor is benign or malignant. The appearance of cancerous cells can range over a broad spectrum, and the line separating benign from malignant is not clearly drawn. A tumor that looks benign may behave in a malignant fashion, or vice versa. But usually it is possible to determine if a tumor is malignant or not, by the appearance of cells in the tumor mass. If tumor cells resemble cells in the presumed tissue of origin, the tumor is likely to be benign. A malignant tumor is likely to be composed of anaplastic cells, showing pathological changes of chromosomal structure and cellular appearance.

The extent of tumor progression is determined by the degree of local tissue invasion and the extent of tumor metastasis. Local invasion occurs when a tumor that began to grow in a particular organ spreads beyond that organ and extends into adjacent structures. Tumor metastasis occurs when cells from a tumor mass move away from that tumor and seed themselves into tissues that are at a distance from the tumor. The tissues most likely to be affected by metastasis are the lymph nodes nearest the tumor. Normal tissues are perfused by a fluid that leaks out of the circulatory system and suffuses through cells.

This fluid is drained from tissue by the lymphatic system, which ultimately returns lymph fluid to the circulatory system. The lymphatic system is comprised of channels that penetrate through tissue, much the way that blood vessels penetrate through tissue. But lymph channels are in intimate contact with cells, and lymph channels lack the protective lining of cells found in blood vessels. Consequently, the lymphatic system often serves as a thoroughfare by which metastatic tumor cells are disseminated to other part of the body. Lymph nodes are simply the gatekeepers of the system, serving a sort of filtration function. Because of this, tumor cells frequently lodge in lymph nodes and may begin to grow there, forming a regional metastasis.

Metastasis is usually the single most important factor in determining the prognosis of a patient. In breast carcinoma, patients with localized disease and no evidence of lymph node involvement can anticipate a 5-year survival rate of about 82%. When one to three regional lymph nodes are found to be cancerous, the 5-year survival rate drops to about 50%. When four or more regional lymph nodes are involved, the 5-year survival rate is only 21%. About 30 to 45% of breast cancer patients have lymph node involvement at the time of diagnosis, and any tumor larger than 2 cm in diameter is already likely to have metastasized, at least to regional lymph nodes.

TUMOR STAGING

An elaborate system has been developed to precisely describe the progression of an individual tumor. This system is usually modified slightly for each type of cancer, but a general overview of the system is provided here. This system was developed in order to assist oncologists in planning a treatment, to provide broad categories for estimating prognosis and evaluating treatment, and to facilitate the exchange of information between physicians concerned with the care of a patient. The system categorizes an individual tumor using a three-variable classification. The three variables are T, N, and M, where T defines the primary tumor, N the extent of lymph node involvement, and M the presence or absence of distant metastases. Use of the system to define the extent of malignant disease is called staging.

The primary tumor (T) category has four subcategories, with criteria as follows. T₁ defines a tumor that is small (usually less than 2 cm in diameter) and confined to the tissue of origin (Fig. 6). A T₁ tumor may be spreading along surface layers of tissue, but it is not deeply invasive, and not attached firmly to

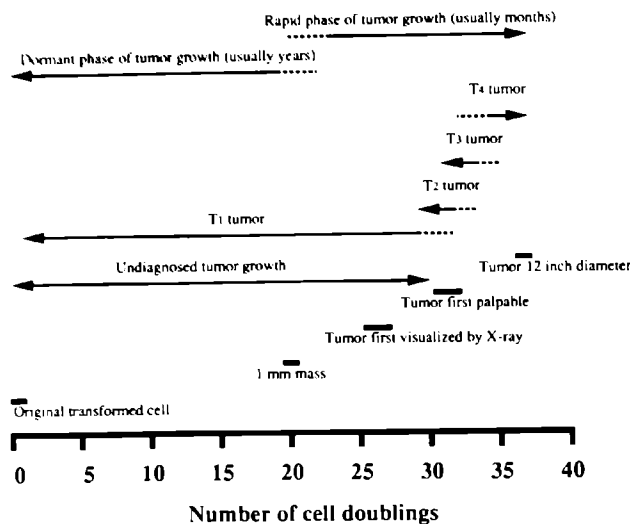


Figure 6. A schematic representation of the growth pattern of a human tumor, with clinical stages indicated. Note that most tumors have completed more than two-thirds of their total growth (roughly 32 doublings) at the time of diagnosis. (From V. T. DeVita, 1987, *Harrison's Principles of Internal Medicine*, edited by E. Braunwald, *et al.* Reprinted by permission of McGraw-Hill, Inc.)

any structure. A T_2 tumor is a localized tumor of 2 to 5 cm in diameter, which is invasive to adjacent tissues or structures. If this tumor is located in a hollow organ, it cannot obstruct more than half of the lumen diameter and it is movable upon palpation. A T_3 tumor is usually 5 to 10 cm in diameter, may be invasive through muscle into adjacent structures, may occlude more than half the diameter of a hollow organ, and may not be movable upon palpation. A T_4 tumor is larger than 10 cm in diameter, is invasive to adjacent organs, may be destructive of those organs, and may be eruptive to the body surface.

The lymph node (N) category has five subcategories, with criteria as follows. N_0 defines a tumor with no evidence of lymph node involvement. An N_1 tumor has local nodal involvement, but the node is solitary, small (2 to 3 cm in diameter), and mobile, signifying that the node has not attached itself to any underlying structures. An N_2 classification is used for tumors with several involved local nodes that are large (3 to 5 cm in diameter), firm, and partially invasive to underlying muscle. These nodes may form matted clumps that are no longer freely mobile. An N_3 classification indicates multiple nodal involvement, where individual nodes are large (5 to 10 cm in diameter), immobile, and invasive to surrounding bone, muscle, or skin. An N_4 classification is reserved

for tumors with extensive involvement of local and distant lymph nodes, where individual nodes are large (more than 10 cm in diameter) and may be destructive of adjacent tissues.

The metastasis (M) category also has five subcategories, with criteria as follows. M_0 describes a tumor without any evidence of metastasis. An M_1 tumor has a solitary metastasis in one other organ, while an M_2 tumor has multiple metastases in one other organ, but with minimal functional impairment of that organ. A tumor classified as M_3 has multiple metastases in multiple organ sites, but the impairment resulting from these metastases is still minimal to moderate. An M_4 classification is reserved for tumors that have multiple metastases in multiple organ sites, where the metastases cause moderate to severe functional impairment. The category M_+ is sometimes used to signify a tumor with any level of metastasis greater than M_0 .

Although exact criteria for staging a particular tumor will vary depending on the site and type of tumor, staging categories can be broadly generalized as follows⁶:

- Stage I (T_1, N_0, M_0): The primary tumor is limited to the organ of origin and there is no evidence of nodal involvement or distant metastasis. A tumor of this stage can usually be removed by surgical resection, although the tumor may recur locally. Long-term survival varies with the particular type of tumor, but is generally from 70 to 90%.
- Stage II (T_2, N_1, M_0): The primary tumor has spread into surrounding tissues and those lymph nodes immediately draining the area of the tumor ("first-station" nodes). The tumor is operable, but because of extensive local spread, it may not be completely resectable and is likely to recur locally. Survival is generally 45 to 55%.
- Stage III (T_3, N_2, M_0): The primary tumor is large, with fixation to deeper structures. First-station lymph nodes are involved, and these may be more than 3 cm in diameter and fixed to underlying tissue. The tumor is not usually completely resectable and survival is 15 to 25%.
- Stage IV (T_4, N_3, M_+): The primary tumor is very large (> 10 cm in diameter), with extensive invasion of adjacent tissue and multiple nodal involvement. There is evidence of distant metastasis and the tumor is often inoperable. Survival is less than 5%.

Clearly, clinical staging is not an exact science. Without exploratory surgery, it can be difficult to accurately estimate the size of a primary tumor that is present deep in the body. Nodal involvement is usually easy to determine in a

late-stage tumor, but involved nodes may not be palpable in an early stage tumor. Finally, it is often impossible to know whether metastases exist, since these metastases may be only a few cells hidden at an unknown site elsewhere in the body.

This description of tumor staging suggests that the way to treat cancer most effectively is to diagnose it before there is nodal involvement or distant metastasis. Early diagnosis, at a time when tumor therapy may be more effective, is the most important reason for an increasing survival rate for certain cancers. For example, the recent increase in long-term survival rate of cervical cancer patients probably has more to do with early diagnosis (because of Pap smear screening) than it does with the actual success of treatment for cervical cancer. In those cancers that cannot presently be successfully treated, early diagnosis will increase the time span between diagnosis and death. This can lead to the mistaken impression that survival time is actually improving for a particular cancer.

CHAPTER 2

Tumor Growth

Any conspiracy is a collection of separate lives bound together in a common struggle for the resources held by the larger body from which the conspiracy grew. The success of a conspiracy is determined by the collective success of the conspirators. In the same sense, any tumor is a mass of separate cells, and the life history of the tumor is determined by the collective life history of its separate cells. If the tumor is able to grow, it is only because at least a portion of the tumor cells are able to survive and proliferate within the tumor mass. Just as a conspiracy must be understood in terms of the politics from which it grew, a tumor must be understood in the context of those cells that gave rise to the tumor. Therefore, we must first have a general understanding of the life history of individual cells before we can understand the growth and development of a tumor.

THE CELL CYCLE

The human body is composed of roughly 75 trillion (7.5×10^{13}) cells, each of which has a life cycle of its own. An individual cell is formed when a single progenitor or mother cell divides, forming two identical daughter cells. The two daughter cells can then go their own ways, growing and differentiating separately, and often following different life cycles. Each daughter cell may be capable of dividing again, forming two more daughter cells, or the daughter cells may fail to undergo further divisions. Certain cells appear to undergo terminal differentiation, so that they may be capable of living for years but are unable to divide again to form daughter cells.

A series of cellular events make up the cell cycle. These events include the creation of new daughter cells by division of a mother cell, the development of those daughter cells into mother cells themselves, and the division of each new

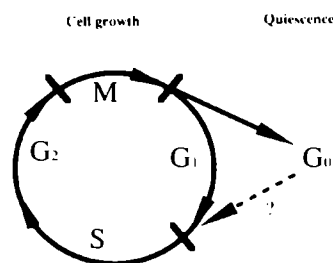
mother cell to form more daughter cells. The division process during which a new generation of cells is formed is called mitosis. Thus, mitosis represents the end of the life cycle of the mother cell, and the beginning of the life cycles of the daughter cells.

During the process of mitosis, a mother cell first replicates its own genetic information, which is contained in the chromosomes. The mother cell then apportions replicated chromosomes equally between the two daughter cells. If, for any reason, a daughter cell does not receive an exact replicate of the chromosomes from the mother cell, the daughter cell is unlikely to survive. Thus, the mitotic process ensures that each daughter cell has all of the genetic information necessary to live. Mitosis amounts to an exquisitely precise choreography of chromosomes as they are apportioned between the daughter cells. The actual events that occur during mitosis are not discussed in detail here, but any biology text can be consulted for a detailed description of the process.

The various stages in the life cycle of a cell have been named and studied, and a great deal of current research is devoted to determining the factors that regulate progress of a cell through the cell cycle. Shortly after a daughter cell is created by mitosis of a mother cell, the daughter cell is in what is called a gap (or G) phase. Although gap phase may seem to denote a time when nothing much is happening, it actually represents the span of time during which each cell is going about its business. That business is different for each different cell type, and it is the combination of thousands of different cell types that allows the human body to do the work involved in living.

At some point, a daughter cell undergoes the process of replicating its genetic information to become a mother cell. Since the genetic information is stored in DNA molecules carried by chromosomes, replication of the genetic information involves synthesis of two exact copies of this DNA. This process of DNA synthesis occurs during synthesis (S) phase of the cell cycle. Following S phase the cell goes through a second brief gap phase, during which it is preparing to undergo cell division. Since there are two gap phases, one preceding and one following S phase, each has been named to avoid confusion. The gap phase preceding S phase is called G_1 , while that following S phase is called G_2 . The duration of G_1 , S, and G_2 can vary greatly from one cell to another, and the various ways that a cell regulates its progression through these phases will be discussed later. At the end of G_2 , the mother cell divides, forming two daughter cells by mitosis. Since the period of time during which a cell is in mitosis also represents a distinct period in the life cycle of the cell, this

Figure 7. Simplified diagram of the cell cycle, showing the normal progression from mitosis (M), to gap phase-1 (G_1), to DNA synthesis (S) phase, to gap phase-2 (G_2), and back to M phase. During G_1 or rest phase the cell has not yet begun to prepare for another round of cell division. Synthesis of DNA in S phase marks the initiation point of another round of cell growth, while in G_2 phase the cell is making final preparations for mitosis. During M phase the cell undergoes division, thereby completing the cell cycle. Also shown is the entry point to a state of cellular quiescence (G_0), and a hypothetical exit point from G_0 , indicating that cancer cells are probably capable of reentering the cell cycle after quiescence.



period has been called mitosis (M) phase (Fig. 7). It is during M phase that the choreography of chromosomal movement occurs, with the goal of producing two daughter cells with identical genetic information.

Certain cells cease to divide, either because they have differentiated to a nondividing cell type or because they have not received the signals that induce a cell to progress through the cell cycle. When a cell ceases to divide, it is usually stuck in what resembles G_1 phase. Since a cell that remains in G_1 for an extended period of time is different from a cell progressing through G_1 , a cell stuck in G_1 is said to be in G_0 phase. This simply denotes that the cell is no longer in the cell cycle. A resting cell in G_0 phase may be capable of reentering the cell cycle in order to divide again. Such a cell thus leaves G_0 and returns to the G_1 phase of the normal cell cycle.

CELL GROWTH RATE

At any given time the proportion of cells actually undergoing division is quite small, either in a tumor or in a normal tissue. If this were not so, a phenomenal cell growth rate would be possible. For example, some bacteria are capable of dividing to produce two daughter cells every 20 minutes. If a single bacterium underwent repeated cell divisions, and all of the progeny of that bacterium were able to grow and divide every 20 minutes, then an astronomical number of cells would be produced in a short period of time. In fact, if this bacterial cell divided at a maximum rate for a single day, it could give rise to about 2,400,000,000,000,000,000,000 cells (2.4×10^{21} or 2.4 billion trillion cells). If this hypothetical cell was only able to divide every hour (instead of

every 20 minutes), it would still produce more than 8 million (8×10^6) progeny in a single day. Clearly, this does not happen, because of resource limitations for the cells.

The proportion of cells undergoing mitosis in a human tumor varies, depending on the particular tumor, but it is generally quite small. A tumor can be surgically removed and examined by microscope, to determine the proportion of cells actually caught in the middle of cell division. The number of cells dividing is called the mitotic index. In a wide range of human tumors, the mitotic index is often less than 1%. For example, ovarian carcinomas have a mitotic index of 0.8–1.2%, while stomach carcinomas have a mitotic index of 1.0%.⁷ Other tumors can have a mitotic index substantially less than 1%, including cervical carcinoma, which has a mitotic index of only 0.3%. But the mitotic index can give a misleading impression that a tumor is growing slowly if tumor cells progress rapidly through M phase. For this reason, the labeling index is usually preferred as a measure of cell growth rate.

The labeling index is a measure of the proportion of cells that become labeled with radioactivity after cells are supplied with a radioactive precursor of DNA. This is equal to the proportion of cells that undergo S phase in the interval between when cells take up the radiolabel and when the tumor is excised and analyzed. Radioactive cells can be at any point in the cell cycle, from S phase, through G₂ phase, to M phase. If cell mitosis is rapid, then the labeling index of a tumor could be much higher than the mitotic index of that tumor. Thus, the labeling index is probably superior to the mitotic index for estimating the growth rate of a population of cells. If ovarian carcinomas are analyzed 1 hour after injection of radiolabel, they have a labeling index of 18–20%, whereas the mitotic index of these tumors was only 0.8–1.2%.⁷ Stomach carcinomas, which have a mitotic index of 1%, have a labeling index of 27–28%. Similarly, cervical carcinomas, which have a mitotic index of 0.3%, can have a labeling index as high as 40%. More recent data, derived from injection of radioactive thymidine into patients with small-cell lung carcinoma, have shown that about 14% of the cells in these relatively slow-growing cancers are labeled.

In many tumors it is thought that the fraction of cells actually able to undergo mitosis is rather small. Cells that can divide and produce progeny are called clonogenic cells (Fig. 8) Estimates of the clonogenic cell fraction have been made from excised tumors by separating the tumor cells and trying to grow them in culture. However, this technique gives an underestimate of the clonogenic fraction, since some cells that could have divided in the tumor may

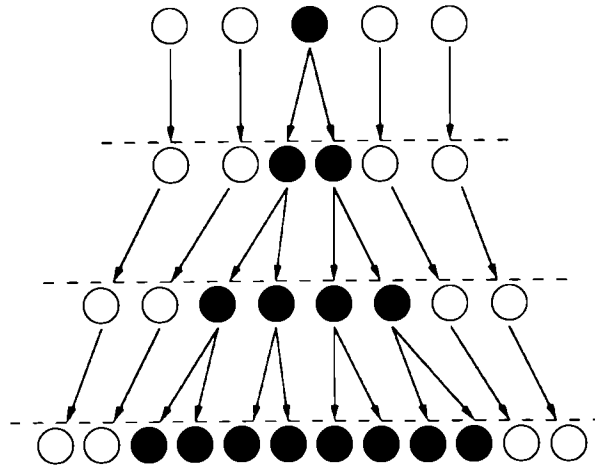


Figure 8. This figure (and the following three figures) illustrate how mitotic index, clonogenic capacity, cell cycle time, and cell loss rate interact to produce a growing population of cancer cells. The filled circles represent clonogenic cells, which are capable of undergoing mitosis to produce two viable progeny cells, while the empty circles represent cells that are not clonogenic (do not produce progeny) but nevertheless survive for a length of time equal to a cell generation. The vertical distance between the dashed lines represents the cell cycle time; lines closer together could indicate a shorter cell cycle time. In this figure the tumor initially has a low proportion of clonogenic cells (20%), but since there is no loss of cells or of clonogenic potential over time, the proportion of clonogenic cells increases rapidly to 67%.

be unable to divide in culture. The tumor cell growth fraction has also been estimated by injection of a radiolabeled precursor of DNA, followed by a long delay before excision of the tumor. Experiments like this show that the tumor cell growth fraction in carcinomas can range from 20 to 100% (Fig. 9). For example, an endometrial carcinoma had a growth fraction of 20%, ovarian carcinomas had a growth fraction of 70–77%, and a stomach carcinoma had a growth fraction of 100%. Furthermore, the tumor cell growth fraction potentially can change over time (Fig. 10).

Cell growth rate is determined by several other factors beyond simply the proportion of cells capable of dividing. The length of time for a cell to progress through the cell cycle has a strong effect on cell growth rate, as we saw in the example of the hypothetical bacterium. Cell cycle time is an extremely difficult parameter to measure, since cells are microscopic and cannot usually be seen in the living organism. Nevertheless, cell cycle time has been estimated for certain rapidly growing human cells using a variety of different techniques.

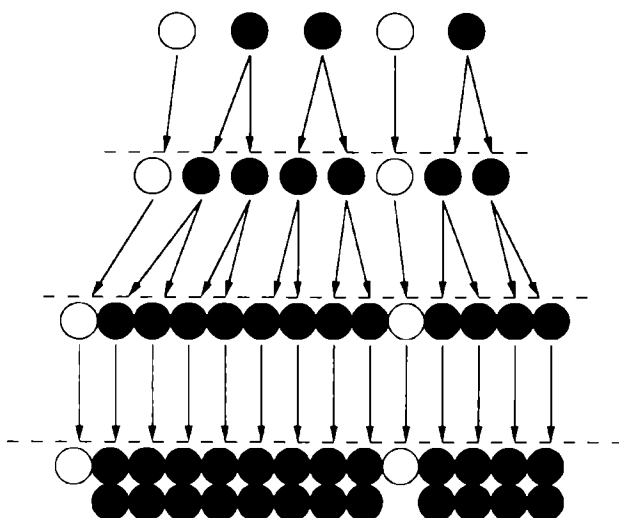


Figure 9. This figure shows a tumor in which the proportion of clonogenic cells is initially high (60%) and the cells grow with a moderately short cell cycle time. Because there is no cell loss or loss of clonogenic potential, the cell population expands very rapidly and the proportion of clonogenic cells quickly becomes very high (92%).

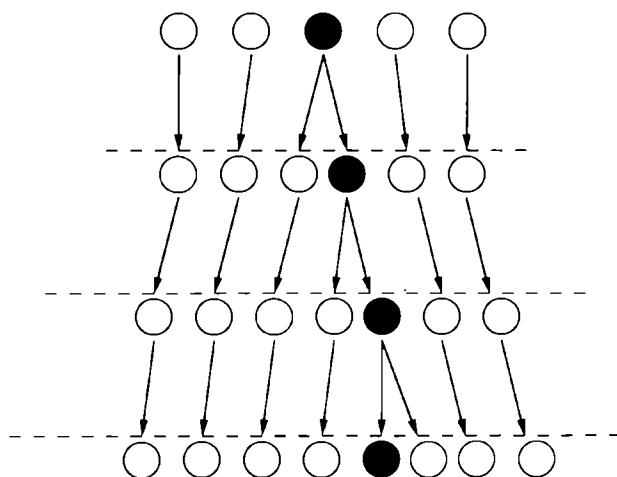


Figure 10. This figure shows a tumor in which the proportion of clonogenic cells is initially rather low (20%), the cell cycle time is moderate, and there is a 50% rate of loss of clonogenic potential by clonogenic cells. Because half of the clonogenic cells produced lose their clonogenic potential, the population of cells in the tumor grows slowly and the proportion of clonogenic cells declines over time to only 13%.

The cycle time of normal cells in the colon has been estimated to be about 39 hours, while normal rectal cells proceed through the cell cycle in about 48 hours.⁷ The rapidity with which these cells go through the cell cycle is unusual, and accounts for why these cells are vulnerable to damage from the chemotherapeutic agents given to cancer patients. Often, chemotherapy is toxic only to cells in a certain phase of the cell cycle. Since cells in the colon progress rapidly through the cell cycle, more of these cells go through the vulnerable phase during the time when the drug is present. The nausea that many patients experience during chemotherapy is caused by selective toxicity of the chemotherapy to cells in the intestines. Estimates of cell cycle time are more difficult to make for cells that grow slowly, so the cycle time for many normal cells is unknown.

Cell cycle time has been estimated in a range of human tumor types. The cell cycle time of a series of carcinomas was estimated to be between 72 and 110 hours, while that of a leukemia was 95 hours.⁷ Since these cells are proceeding through the cell cycle at roughly the same rate as normal intestinal cells or bone marrow cells, the tumor cells are likely to be no more vulnerable to chemotherapy than the normal cells. In fact, normal cell toxicity sets the upper limit on how much chemotherapy can be administered to a patient. While it might be possible to cure more tumors if extremely high doses of chemotherapy were used, the "cure" might kill the patient. Typically, chemotherapeutic agents are given at a dose at which toxicity to normal tissue is the limiting factor.

The proportion of cells that survive after division will also have a strong effect on cell growth rate (Fig. 11). It is easy to see that if cells were to suffer a 50% mortality rate after cell division, then a single cell could not give rise to any progeny, no matter how rapidly it divided. The actual rate of cell survival after mitosis is essentially impossible to determine, because cells are so small and cannot be individually tracked. But there are several reasons to expect a high rate of cell loss or cell death in tumors. Tumor cells can be lost from the tumor because of their inability to tolerate the microenvironment within the tumor, through migration or metastasis of cells out of the tumor, through attack on the tumor cells by the host immune system, and through the inability of genetically damaged tumor cells to survive.

The cell loss rate in tumors is thought to be quite high, because there is often a discrepancy between the calculated rate of cell growth and the measured rate of tumor growth. It has been estimated that between 50 and 80% of all newly produced tumor cells go on to die, meaning that the mean cell loss rate is 25 to 40%.⁷ Other estimates have placed the mean cell loss rate as high as 45%.

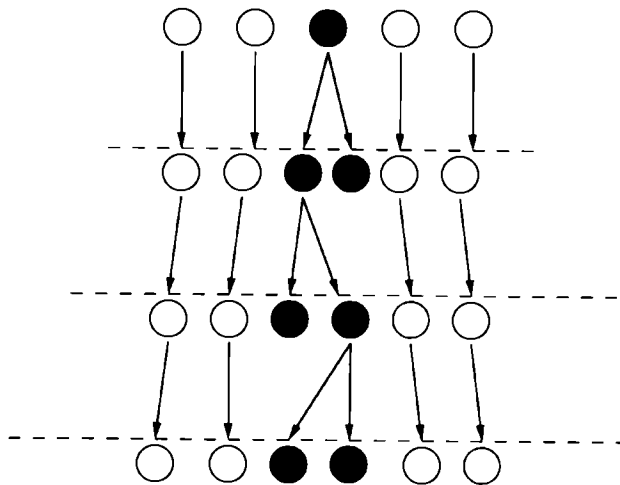


Figure 11. This figure shows a tumor in which there is a low proportion of clonogenic cells (20%) and a high rate of loss of cells from the tumor (lost cells shown in gray). If these cells are lost because of cell death, the growth of the tumor will be quite slow. If these cells are lost because they become metastatic, the growth rate of the primary tumor may be quite slow, but the rate of establishment of secondary tumors may be very high. Note also that the proportion of clonogenic cells in the tumor does not change over time.

Cell loss rate is one of the dominant factors in determining how fast a tumor is able to grow, since tumor growth is nothing more than the net balance of cells remaining after production and loss of new cells (Fig. 12).

NORMAL AND CANCER CELL GROWTH IN CULTURE

Often, cells from the body of an animal or a person can be brought into cell culture, so that they grow and divide independently of other cells of the body. Growth in cell culture is only possible when the requirements of the cells are well understood, because the culture medium is performing the normal function of blood in supplying nutrients. When cells are brought into cell culture, it is usually found that normal cells grow in a very different way than cancerous cells.

Typically, normal (noncancerous) cells growing in a culture dish will grow until they carpet the bottom of the culture dish. A uniform monolayer of cells is formed as the normal cells continue to grow, but eventually the cells cease to grow when they encounter one another. This inhibition of cell growth actually

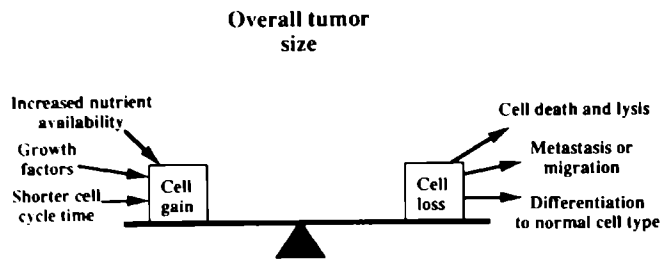


Figure 12. Schematic diagram showing the effect on overall tumor size of various processes that either increase the rate of cell gain or increase the rate of cell loss. Net tumor size is shown as a balance achieved between processes that tend to increase the production of new cells and processes that tend to increase the loss of old cells.

results from cell-to-cell contact, so it has been called contact inhibition. If contact-inhibited normal cells are removed from a confluent monolayer and are placed in fresh medium, they will soon begin to grow again. This shows that there were no irreversible cellular changes associated with contact inhibition. Contact inhibition is also not the result of cells using up some limiting nutrient in the cell broth surrounding them. If the growth medium of a contact-inhibited cell culture is rejuvenated with fresh medium, normal cells still will not grow.

Cancerous cells in culture seem to lose their ability to respond to neighboring cells, so that they are no longer contact-inhibited. Thus, cancer cells grow to confluence and then just keep growing, forming a piled-up mass of cells. Because cells in the interior of this mass sometimes have limited access to nutrients in the cell medium, some cells in the mass may starve and die. Occasionally, cancer cells growing in culture will actually detach from the walls of the culture vessel and grow floating free in the culture medium. Such cells can form balls or irregular clumps of cells. This type of growth morphology is never seen in normal cells.

Normal cells grown in cell culture typically are able to undergo a finite number of cell doublings before they lose the ability to divide. This phenomenon was first noted more than 30 years ago, but the mechanism limiting cell division is still not well understood. In any case, a normal human fibroblast brought into culture is capable of dividing about 50 times to form daughter cells. When fibroblasts are obtained from persons of different ages, those from older individuals are able to undergo fewer divisions than cells from a younger person. If cells are frozen for long periods of time and then unfrozen, they seem to retain some "memory" of the right number of divisions to go through before

they cease to divide. Thus, frozen cells will grow until they have gone through the same number of generations as their unfrozen counterparts, then all of the cells will die after the same number of divisions.

Cancer cells do not show a similar limitation on the number of cell divisions they can undergo. In fact, tumor cells are essentially immortal, capable of undergoing apparently limitless rounds of cell division. The first cancer cells brought into culture were derived from a cervical carcinoma, which was brought into culture in 1951. These cells are named HeLa cells, in honor of Henrietta Lacks, the woman who contracted the original cervical carcinoma and eventually died of her disease. These cells have been in culture more than 40 years, and have undergone untold numbers of cell doublings with no sign of diminished vigor. In fact, HeLa cells still grow so vigorously that they are “lab weeds,” often found as contaminants in cultures of other cells.

Normal cells grown in culture typically tend to resemble the cells from which they are derived. Thus a skin cell in culture will look distinctly like other skin cells, whether in culture or in the individual, while a muscle cell will look distinctly different. Normal cells in culture tend to retain some degree of differentiation that sets them apart from cells derived from other tissues.

Cancer cells grown in culture will often look completely unlike the tissue from which they are derived. Although some tumor cells retain a degree of cellular specialization, most lose the obvious features associated with differentiated cell function. Certain cancer cells retain no differentiating features at all, so that it is essentially impossible to tell the tissue of origin of the cancer cells. This loss of cellular differentiation is typically more pronounced in cells that grow very rapidly in culture. In fact, there appears to be a fairly close inverse relationship between the rate of cell growth and the degree of cellular differentiation. This is true even in a tumor, since rapidly growing tumors are often masses of completely undifferentiated cells.

THE KINETICS OF TUMOR GROWTH

We have seen that the growth rate of a tumor depends on three factors:

1. The average duration of the cell cycle time
2. The fraction of cells capable of undergoing mitosis
3. The proportion of cells that survive after mitosis

Generally, the rate of tumor growth can be estimated far more easily than the rate of growth of individual cells. Tumor growth rate is usually estimated by measuring the volume of a tumor at two different points in time. In order to characterize the maximal tumor growth rate, the tumor should not be treated in the interval between the two measurements, or else the measurement will be complicated by the death and removal of some tumor cells and the regrowth of other cells. Clearly, it is unethical to leave a tumor untreated merely to satisfy the curiosity of scientists, so few such measurements have been made. The only time such measurements have been made is when tumor treatment is inadvertently delayed. This can occur because a patient is in a weakened condition and cannot tolerate treatment, because the tumor is progressing very rapidly, or because the patient refuses treatment. Since these conditions are unusual, it is unknown to what extent the tumor growth measurements that have been made are representative of the growth rate of tumors.

In any case, tumor growth rate can vary dramatically from one tumor type to another. Data compiled from a number of different researchers have been used to examine the relationship between tumor growth rate and tumor response to therapy.⁷ Lung tumors metastatic from a highly malignant bone tumor (Ewing's sarcoma) had a doubling time of only 18 days. A series of lymph node metastases showed an average tumor volume doubling time of 27 days. At the other end of the spectrum, primary adenocarcinoma of the colon and rectum was estimated to have a doubling time of 632 days and bronchial adenocarcinoma 148 days. For most tumors examined, volume doubling fell in the range of 50 to 100 days.

Despite the fact that different tumor types grow at different rates, the growth rate of a given tumor type can span a broad range. Data have been compiled from 159 lung tumors, all of which were metastatic from primary carcinomas at sites throughout the body.⁷ Tumor doubling time for adenocarcinoma metastases varied from 10 to 1000 days. The probability of a lung adenocarcinoma doubling in 10 days or less was only about 1%, but there was also only a 1% chance of the tumor having a doubling time greater than 500 days. There was a 50% probability that a lung metastasis of adenocarcinoma would have a doubling time less than 79 days.

These studies of tumor growth rate have established several concepts that are important in the clinical management of the cancer patient. Most importantly, there is often a long period of tumor growth during which the patient is free of cancer symptoms. This is particularly true before the initial diagnosis of cancer is made: the growth rate of some tumors is so slow that a patient may

harbor a tumor for many years before it is diagnosed. In patients who undergo surgery to remove a primary tumor, it is generally expected that there will be a symptom-free period following surgery, even if all of the primary tumor was not excised. The failure to detect metastatic disease until many months after surgery to remove a primary tumor is probably a function of the slow growth rate of these tumors, and not related to suppression of tumor growth by the immune system of the patient. Generally speaking, the more malignant a tumor, the faster the tumor growth rate is, and tumors with a short volume doubling time tend to cause more rapid decline of the patient.

CELL GROWTH FACTORS

Many normal cells retain the capacity to grow and divide throughout the human life span. This is critical because of the ongoing need of the body to heal itself after injury, or to replenish those cells that die in the normal course of events. Control or regulation of cell growth is essential for normal functioning of the adult body, or for growth and maturation of the child.

The proliferation of all cells, even tumor cells, appears to be controlled by growth factors. A wide range of growth factors, which are usually proteins, can stimulate or repress cell growth rate. Growth factors are secreted by cells or organs of the body in a very tightly regulated manner during normal cell growth. For example, normal skin cells are stimulated to grow during the wound healing process, to replace cells lost or damaged at the wound site. Yet they must also be told when to stop growing; faulty regulation of skin cell growth can sometimes be seen in the clinic, when a reddened, protruding scar forms along the margins of a healed surgical incision. These scars, called keloids, represent a failure of cell growth regulation: they result from over-production of collagen, a normal constituent of skin cells.

Another interesting example of cell growth regulation can be found in tissue regeneration. The normal growth rate of cells in the adult liver is negligibly low. Yet up to 80% of a rat liver can be surgically removed and the remaining liver cells will regenerate a new liver of normal size in about a week. Within an hour of surgery, changes are seen in the remaining liver cells indicating that the cells are preparing to divide. One day after surgery, liver cells begin to divide to replace the lost liver mass. Liver cell growth continues until the lost liver mass is replaced, then cell growth slows and stops. Partial

liver regeneration has even been documented in patients who have undergone surgery to remove a tumorous portion of their liver.

If cells lose the appropriate inhibitory response to certain growth factors, this will lead to malignancy as surely as if cells acquired an abnormal stimulatory response to growth factors. A good example of this is seen with the growth factor called transforming growth factor, type β (TGF- β). TGF- β is a protein that stimulates the growth rate of skin fibroblasts in culture and causes these cells to behave as if they were transformed to the cancerous state. Yet TGF- β is actually inhibitory to the growth of most cells in culture. Loss of responsiveness of cells to the growth-inhibiting action of TGF- β may be associated with the uncontrolled cell division of cancer. One tumor line has been described that lacks detectable quantities of the membrane receptor to which TGF- β binds, so that these tumor cells are unable to respond to TGF- β . In fact, abnormal production of growth factors, or abnormal response to growth factors, appears to be involved in the formation of many, if not most, cancers.

REGULATION OF PROGRESS OF CELLS THROUGH THE CELL CYCLE

Growth factors are involved in the manifestation of many different cancers because they regulate the rate at which cells progress through the cell cycle. Some growth factors apparently stimulate cells to emerge from the quiescent state (G_0) and reenter the cell cycle, while other factors may be required for cells to progress normally through G_1 and enter S phase. After a cell has entered S phase, additional growth factors do not appear to be required. Cells in culture can progress through S, G_2 , and M phase in the absence of any growth factors. But when cells again reach G_1 phase, they require exogenous growth factors to continue cycling.

The cellular events that regulate progress through the cell cycle are very complex. There is a critical event in G_1 phase, called the restriction point, that cells reach under the influence of a growth factor called epidermal growth factor (EGF). Once cells pass the restriction point, they are committed to continue through the cell cycle to mitosis. A cell can pass the restriction point if it can accumulate enough of a particular protein called restriction point protein. However, accumulation of a sufficient quantity of restriction point protein is difficult, because the protein is unstable and breaks down continually. Unless a

cell is rapidly synthesizing the protein, it cannot accumulate enough to pass the restriction point, so the cell essentially becomes locked into G_1 phase.

Quiescent (G_0) cells are quite different from G_1 cells that are not proceeding through the cell cycle. Cells in G_0 and those in G_1 both have unreplicated DNA, but G_0 cells also lack the cellular machinery needed for protein synthesis. Before G_0 cells can reenter G_1 , they must reactivate their protein synthetic machinery. Cells in G_1 already have all of the necessary protein synthetic machinery in place. Cells in G_0 can reenter G_1 under the control of certain growth factors, while G_1 cells are already receptive to the growth factors that stimulate a cell to pass the restriction point. Thus, when G_1 cells accumulate the appropriate amount of the restriction point protein, they can rapidly enter S phase.

Cancer cells appear to have a relaxed requirement for growth factors and are able to pass the restriction point more easily than normal cells. In some cancer cells the restriction point protein appears to be stable, so that it accumulates more readily. In other cancer cells, accumulation of the restriction point protein is not as sensitive to external factors as in normal cells. In either case, cancer cells can divide under conditions that would not permit division of normal cells. This explains why, for example, liver tumor cells can divide whereas untransformed liver cells normally are unable to divide.

THE CLONAL ORIGIN OF CANCER

There is accumulating evidence that most human tumors originate from a single mutated cell, so that the tumor represents a clone of a single progenitor cell. Evidence in support of this conclusion has come from several different sources. In many primary tumors, all of the cells share a chromosomal abnormality that is absent from normal cells of the same individual. For example, in patients with chronic myelocytic leukemia, all cancerous cells have the Philadelphia chromosome (Fig. 2), whereas this abnormality is not seen in normal cells from the same patient.

Another piece of evidence suggesting that tumors are clonally derived from a single cell has come from studies of multiple myeloma. Multiple myeloma is a malignant proliferation of plasma cells, which ordinarily give rise to B cells in the immune system. Since each B cell normally secretes a different antibody, one would expect that a large population of cancerous B cells would secrete a large number of different antibodies. But all of the cells of a multiple

myeloma usually secrete the same antibody, suggesting that each cell was derived from a single progenitor cell.

Another piece of evidence suggesting that tumors are clonally derived has come from a study of gene expression in tumor cells removed from female cancer patients. All females have two X chromosomes, so two different versions of a particular gene can potentially be carried on the two X chromosomes. Since only one X chromosome is needed for an individual cell to survive, each cell of a woman's body randomly inactivates one of the two X chromosomes. Because inactivation is random, there is a 50% probability of inactivating a given X chromosome. In a large population of cells, this means that half of the cells will express whatever genes are on one X chromosome, while half of the cells will express genes from the other X chromosome. Therefore, the cells of a woman's body are a mosaic of cells expressing two different versions of all X-linked genes. Yet a study of gene expression in the cells of a tumor mass has shown that all cells in the tumor express the same gene. Since this gene is carried on the X chromosome, this indicates that all of the tumor cells have inactivated the same X chromosome, an event that is highly unlikely to occur by chance. In fact, if there are 10^8 cells in a tumor mass, the chance that all cells of the tumor would be identical in the absence of shared ancestry is only 1 in 100 million. This strongly suggests that all cells of the tumor mass were derived from a single cell.

CLONAL EVOLUTION IN CANCER

If cells in a tumor are always derived from a single progenitor cell, this would imply that all cells in a tumor are always genetically identical. In fact, this is not true. Tumor cells appear to be genetically more unstable than normal cells, although the reason for this instability is unknown. It is easy to show in cell culture experiments that tumor cells have a higher rate of spontaneous mutation than normal cells, and that the mechanisms designed to repair damaged DNA are often less effective in tumor cells than in normal cells. The genetic instability of cancer cells can actually cause these cells to undergo a process of evolution as they grow to form a tumor.

The fact that tumor cells divide continuously can lead to production of a large number of new cells in a short period of time. However, the rate of survival of newly produced cells is often very low. Whether a particular newly produced cell survives or not is largely a matter of chance. If a cell is produced

in a microenvironment supportive of cell growth, the cell may survive, but if produced in a hostile microenvironment, it will probably die. A cell carrying a mutation that makes it unusually well adapted to a particular microenvironment, however, may survive even though conditions prevent the growth of other tumor cells. If the mutated cell can transmit that mutation to its daughter cells, the daughter cells also will enjoy a growth advantage compared with the other cells of the tumor. This sort of mechanism can potentially lead to the production of multiple clones of new cells, genetically different from each other and from the original cancer cell that produced them. These genetic variants may be better able to grow and divide under the conditions present in the tumor (Fig. 13). This amounts to a process of natural selection for cells that are more successful at producing tumors.

Clonal selection can potentially account for why a tumor occurs in the first place. If normal cells are genetically damaged by a carcinogenic agent, then most of those cells will simply die. However, there may be some cells that can survive and grow. If a carcinogenic agent transforms a cell and induces it to undergo clonal expansion, that cell may produce mutated progeny because of genetic instability. The variant cells may die in large numbers, but conditions are also changing within the tumor as the tumor mass expands. Eventually a mutated cell may be produced that is better able to tolerate the conditions found within the tumor. If a genetically mutated cell is also released from the growth constraints that normally inhibit cell growth, then the new cell could potentially form a tumor. Thus, clonal selection of rare cell types may be common within a

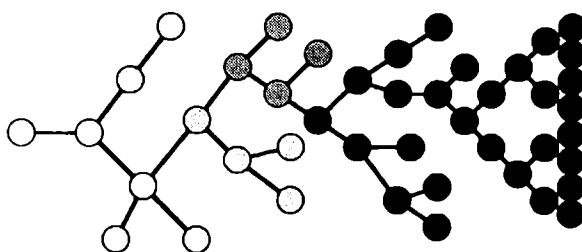


Figure 13. Figure illustrating clonal evolution of cells within a tumor. The shading of the dots suggests an increasing level of malignancy with the passage of time; normal cells are shown as white, while highly malignant cells shade to black. A vertical section through this diagram samples the tumor cell population at any one time. The length of the lines connecting the cells indicates cell doubling time; note that doubling time decreases as the tumor cells evolve to a more malignant form. (From V. T. DeVita, 1987, *Harrison's Principles of Internal Medicine*, edited by E. Braunwald *et al.* Reprinted by permission of McGraw-Hill, Inc.)

tumor because, as the tumor grows, new cellular environments are constantly being created.⁸ Conditions within the tumor become more varied and generally less supportive of cell growth, placing ever more stringent selection pressures on the growing cancer cells. As we will see in the next chapter, many carcinogenic agents are simply mitogens that cause normal cells to grow more rapidly. If these rapidly growing cells also become genetically unstable, then an opportunity is created for cells to mutate and undergo clonal selection. The end result of this process may be that carcinogens simply select for cells with the ability to form a tumor.

Clonal selection may also be able to explain why a tumor frequently can become resistant to therapy as it grows. Many tumors are initially sensitive to a particular form of therapy, but after that therapy has been used several times, the tumor becomes increasingly resistant to it. For example, both chemotherapy and radiotherapy usually can cause cancer remission after initial treatment of small-cell lung carcinoma (SCLC). But SCLC is seldom cured by therapy since some tumor cells survive and cells that do survive are probably inherently resistant to therapy. As the surviving cells divide, they produce progeny that share in the inherent resistance to therapy. If the therapy kills a large number of nonresistant cells, then it would quickly remove vulnerable cells and leave behind resistant ones. Clonal selection of surviving cells can thus account for acquisition of drug and radiation resistance by SCLC tumor cells.

CELLULAR DIFFERENTIATION IN CANCER

Cancer cells are usually able to divide continuously and they may appear to be structurally simpler than normal cells. Furthermore, many different cancer cells synthesize substances that are characteristic of fetal tissue (e.g., carcinoembryonic antigen is synthesized by colorectal cancer). This led to the belief that cancer cells are locked irreversibly into an immature state that enables them to divide continuously, much like the undifferentiated state of cells in a fetus. According to this notion, cancer results when cells are unable to undergo cellular differentiation to form mature tissues. Recently this interpretation has been called into question because it appears to be an oversimplification of what is really happening in a tumor.

The idea that cellular differentiation is something that is irreversibly lost by tumor cells is apparently untrue. Many tumors are comprised of cells that

are at least partially differentiated. For example, colon adenocarcinomas typically are composed of glandlike structures that resemble glands found in the lining of the normal colon. The glandlike structures in colon cancer even form mucus, as normal colon cells do, and the cells retain the ability to form glandlike structures even when brought into cell culture. Furthermore, certain undifferentiated cancer cells can be induced to differentiate into apparently normal tissues under specific conditions. Recently there have been several clinical trials of agents designed to induce cancer cells to differentiate, so that the cells lose their ability to proliferate continuously. For example, cytosine arabinoside (Ara-C) is a drug that is already widely used in combination chemotherapy for acute myeloid leukemia. Studies with cultured leukemia cells suggest that Ara-C induces leukemia cells to differentiate when given at a lower dose than is typically used in chemotherapy. Low doses of Ara-C have also been used to treat patients with "preleukemia," a condition in which bone marrow cells fail to mature properly. These patients frequently develop a full-blown case of leukemia and are very vulnerable to infection. Although the initial clinical trial results were somewhat disappointing, about a third of the "preleukemia" patients treated with Ara-C had a complete or partial remission.

Another promising agent for inducing cellular differentiation is a growth factor called granulocyte-colony stimulating factor (G-CSF). G-CSF given to myeloid leukemia cells in culture induces the leukemia cells to differentiate into blood cells called neutrophils. This could potentially be very important in the clinic because leukemia cells cease dividing and form cells that are active against bacterial infection. G-CSF has not been tested in patients, but it has been tested in leukemic mice. G-CSF can extend the life span of leukemic mice from less than 2 months, the life expectancy without treatment, to more than 6 months. Furthermore, this result can be obtained when G-CSF is administered for only 28 days, suggesting that leukemia can undergo remission if all of the replicating cells differentiate.

If tumor cells can be induced to undergo differentiation and cease dividing, this will almost certainly have a major impact in the clinic. A differentiated tumor should be easier to remove by surgery, with less possibility of leaving behind cells that cause tumor recurrence. There is also a possibility that if tumor cells can be induced to differentiate, then the cells may spontaneously die.

CHAPTER 3

The Causes of Cancer

A search for the causes of cancer has occupied physicians and scientists for hundreds of years. In 1700, Bernardino Ramazzini noticed that nuns had a higher incidence of breast cancer than any other group of women. He attributed this increased risk of breast cancer to celibacy, thereby making the first connection between cancer incidence and life-style. The potential of tobacco to cause cancer was first noted in 1761, when John Hill suggested that use of snuff resulted in development of nasal cancers. Somewhat later, in 1775, Percival Pott attributed the high incidence of scrotal cancer in chimney sweeps to their constant exposure to chimney soot.

STUDYING CANCER CAUSATION

The complex series of events that results in the production of a cancer is called carcinogenesis. A great deal of research is done in this field today, in the hope that cancer can be eliminated if the causes are known. Researchers who study the causes of cancer usually use one of two different approaches to the problem. The first approach is to study groups of people in whom the incidence of a particular cancer is either unusually high or unusually low. By determining what genetic, occupational, or life-style factors this group shares, and what factors set this group apart, causes may be found for the abnormal incidence of cancer. The second approach is to experimentally probe the relationship between cancer and a proposed causative agent.

In the first approach, that of looking for the etiology of an established pattern, the search for causes is retrospective. That is to say, the cancer has already occurred before the factors common to the victims are sought. The retrospective (or epidemiological) approach was used in the three early studies described above. While this approach can yield very suggestive results, the

evidence is only circumstantial. The alternative approach to the study of cancer causation is to do a prospective study. In a prospective study, an experiment may be done on an animal model of cancer, or data may be collected from individuals at a point before they become cancer patients. This population of individuals is then followed over time to see which, if any, develop cancer.

The difference between a retrospective and a prospective study is the position of the observer in time, with respect to the observed event. If the observed event, cancer, is in the past, then the study is retrospective. If the observed event is at some point in the future, the study is prospective. This difference is crucial, because if a past event is observed, a researcher can attribute causes but cannot manipulate them. If a researcher is trying to observe a future event, there is more scope for manipulating the factors presumed to be causative and for recording events in an unbiased way.

As an example of the difference between a retrospective and a prospective study, assume that a researcher is interested in causation of lung cancer. The researcher may determine, after interviewing a large number of lung cancer patients and an equal number of people who do not have the disease, that the patients are more likely to be tobacco users. The conclusion could then be that tobacco use causes lung cancer. However, heavy smokers are also more likely to be heavy drinkers. Unless the control population of nonsmokers drank as heavily as the smokers, then one could not determine whether the increased incidence of lung cancer was really the result of tobacco or of alcohol. A naive or misguided researcher might even conclude that alcohol use causes lung cancer. Thus, the real danger of a retrospective study is in finding a spurious correlation between a cancer and a presumed causative factor that actually has nothing to do with that cancer.

In a prospective study, the researcher might wish to test the hypothesis that tobacco use leads to an increased risk of lung cancer. The researcher would test this hypothesis by carefully matching a population of smokers to a population of nonsmokers, with every other variable being constant. Thus, there should be no difference between cases and controls in terms of alcohol use, occupational or environmental exposure to carcinogens, and so on. If the population of smokers then goes on to develop a higher incidence of lung cancer, the researcher can be fairly sure that tobacco use is really the cause of the difference between cases and controls. Alternatively, a researcher might perform an experiment in which an experimental population of animals is exposed to a presumed carcinogenic agent in tobacco smoke. The control population of animals would be handled in the same way, except that these animals would not

be exposed to the carcinogen. If the experimental animals then go on to develop lung cancer at a higher rate than the control animals, this evidence would favor the hypothesis that tobacco use causes lung cancer. The greatest danger of a prospective study is that the initial hypothesis is wrong, so that a great deal of work is done to disprove a hypothesis that was incorrect.

As flawed as our present methods of studying cancer causation are, everything we know about the subject has come from either a retrospective or a prospective study. In many cases a clear correlation has been shown between a carcinogen and a particular cancer, even though the mechanism of action of the carcinogen is unknown.

GENERAL MECHANISMS OF CARCINOGENESIS

Many different causes of cancer have been identified, but most of them share certain similarities. Malignant transformation of cells is usually associated with some mechanism that enhances the normal rate of cell proliferation.⁹ A dividing cell is more at risk of mutating than is a quiescent cell, because cellular DNA is not as well protected during S (synthesis) phase or during cell mitosis. Chronic stimulation of cell division is clearly involved in many types of human cancer, including hormonal stimulation of mitosis in breast and prostate cancer, and viral stimulation of mitosis in cervical cancer. Chemical induction of an increased rate of cell division has been implicated as the basic mechanism of chemical carcinogenesis. This has been shown by studies of skin carcinogenesis after topical application of a cell mitogen. Typically, if the dose level of the mitogen is increased, so that cell mitotic rate is enhanced in a dose-dependent fashion, then the time period before appearance of a tumor is shortened. Thus, the rate of tumor progression is increased if the rate of cell mitosis is increased, since cell division increases the risk of genetic errors of various kinds.¹⁰

Carcinogens may also cause tumors by directly inducing mutations in the cellular content of DNA, or by interfering with the ability of cells to repair damaged DNA. But these effects on cellular DNA cannot produce a tumor in the absence of cell proliferation. This is because the toxic effects of a carcinogen on a cell are limited to a single cell unless that cell continues to grow.

Extensive research in carcinogenesis has shown that the causes of cancer fall into two broad categories: causes intrinsic to the patient and causes

extrinsic to the patient. Intrinsic causes of carcinogenesis include hereditary factors and hormonal factors, while extrinsic causes of carcinogenesis include chemicals, viruses, dietary factors, and radiation.

HEREDITARY FACTORS IN CARCINOGENESIS

For certain relatively common types of cancer, disease incidence is higher in persons with a positive family history than in those without such a history. This is true of breast cancer, colon cancer, prostate cancer, stomach cancer, and possibly several other common cancers. The consistency with which it is found that breast cancer patients have a first-degree relative (e.g., grandmother, mother, sister, aunt, or daughter) with the disease, suggests that a predilection to breast cancer can be inherited with the genes. While the relative risk in such cases is usually not dramatically higher, it can be two- to threefold higher. Yet, while there can be a familial predisposition to cancer, most cancers arise in individuals having no such genetic predisposition.

There are, however, a number of hereditary cancer syndromes, in which there is a very dramatic familial increase in the incidence of certain cancers. Hereditary cancers are more likely to arise at an early age and are also more likely to occur simultaneously at multiple sites. Many of the hereditary cancers are associated with a disorder in which there is initially a benign proliferation of cells, then cells undergo malignant transformation and progress to form a cancer.¹¹

An example of a hereditary cancer syndrome that initially involves a benign proliferation of cells is familial adenomatous polyposis or polyposis coli, in which affected individuals develop innumerable tiny polyps in the colon. A colonic polyp is a mushroomlike growth attached to the wall of the colon by a stalk of tissue and projecting into the intestinal lumen. The tendency to develop colonic polyps is inherited, and is transmitted by a gene that is dominant to the normal gene. Since the gene for colonic polyps is dominant to the normal gene, the mutant gene is expressed even though it is opposed by a normal gene. Thus, about half, and possibly all, of the children of a patient with familial polyposis coli will also have the disease. Although the polyps are initially benign proliferations of epithelial tissue, they tend to progress to malignancy over time. About half of all individuals with familial adenomatous polyposis will develop colon cancer by 30 years of age, and virtually all such patients will have colon cancer by the seventh decade of life. However, despite

the fact that most people with familial adenomatous polyposis develop colon cancer, only about 1% of all colon cancer patients had adenomatous polyposis before they developed cancer.

Another example of a hereditary cancer is retinoblastoma, a cancer of the retina that typically affects children under 3 years old. This cancer is transmitted by a specific gene, recessive to the normal gene, which is responsible for about 40% of all cases of retinoblastoma. A child born without the retinoblastoma gene mutation has only a 1 in 30,000 chance of developing this cancer, while the probability that a child with the mutation will develop retinoblastoma is about 95%. This translates to a 100,000-fold higher risk of developing retinoblastoma in those children with the mutated gene. Yet about 5% of patients who carry the retinoblastoma gene never develop retinoblastoma. Furthermore, retinoblastoma patients typically develop no more than three or four cancers among the millions of retinal cells that have the mutation. Therefore, malignant transformation is a rare event on the cellular level, suggesting that the retinoblastoma gene alone is not sufficient to cause cancer. A two-step model of carcinogenesis has been proposed, which postulates that a second mutational event must occur for a cancer to develop from retinal cells carrying a mutant retinoblastoma gene (Fig. 14). This model will be discussed more fully in the section on multistage carcinogenesis.

Critical insights into hereditary factors in carcinogenesis have been provided by a recessive genetic disorder called xeroderma pigmentosum (XP). XP is characterized by extreme sensitivity of the skin to sunlight, and XP is associated with a greatly increased risk of various skin cancers. The basic problem is an impaired ability of cells to repair the DNA damage induced by exposure to ultraviolet light, so that virtually all XP patients develop some form of skin cancer. In addition, these patients are much more sensitive to chemicals that induce DNA damage. DNA repair ability may be impaired by 50 to 90% compared with normal individuals, and the extent of impairment correlates well with disease severity. XP is perhaps the most direct evidence that genetic mutations can cause cancer.

In addition to familial polyposis coli, there are a number of other hereditary cancer syndromes that are transmitted by a mutant gene that is dominant to the normal gene. These syndromes include: Gardner's syndrome (associated with a very high incidence of colonic adenocarcinoma); palmar-plantar hyperkeratosis syndrome, or tylosis (associated with a 95% incidence of esophageal cancer); neurofibromatosis ("Elephant Man disease," associated with a low incidence of neural tumors); multiple mucosal neuroma syndrome

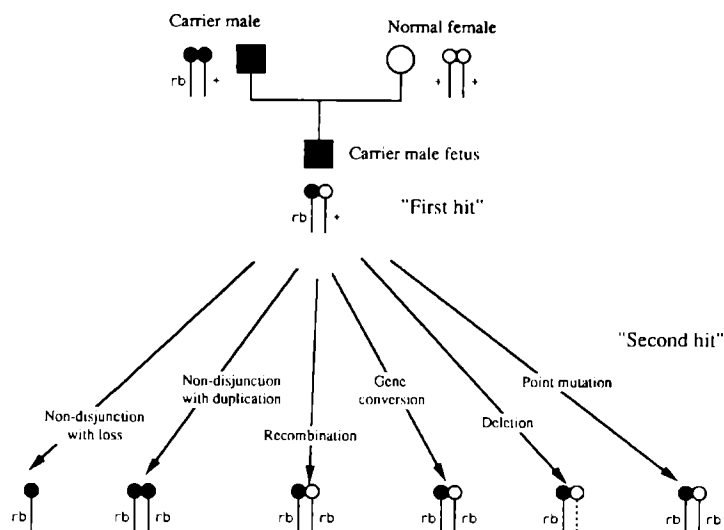


Figure 14. Diagram of the “two-hit” model of expression of the retinoblastoma gene. The male contribution to the fetal genome is shown in black, the female contribution in white. The first “hit” is inheritance of one copy of a mutant form (*rb*) of the wide-type gene (+). The carrier male fetus is vulnerable to a “second hit,” which inactivates, deletes, or otherwise suppresses the one remaining normal (+) gene. Once the abnormal *rb* gene is unopposed by a normal (+) gene, the cell carrying the *rb* gene is transformed to a cancerous state. (From W. Cavenee *et al.*, 1983, *Nature* 305:780 Reprinted by permission of Macmillan Magazines Ltd.)

(associated with a high incidence of adrenal and thyroid gland tumors); and von Hippel–Lindau syndrome (associated with kidney cancer).

Several other familial cancer syndromes are caused by mutation of a gene that is recessive to the normal gene. Cancer syndromes in which the mutated gene is recessive are generally not expressed unless some sort of genetic accident knocks out the function of the dominant normal gene. Recessive cancer syndromes include: Bloom’s syndrome (associated with a high incidence of acute leukemia); Fanconi’s anemia (associated with a high incidence of leukemia and lymphoma); Werner’s syndrome, or adult progeria (a premature aging syndrome associated with a moderate incidence of sarcoma and meningioma); ataxia–telangiectasia (associated with a low incidence of lymphoma, acute leukemia, and possibly gastric cancer); and Turcot’s syndrome (associated with colon cancer and brain tumors). Both Bloom’s and Fanconi’s syndrome involve an inherited instability of the chromosome, and a high frequency of chromosomal rearrangement. The mechanism involved in Bloom’s and Fanconi’s syndrome supports the idea that genetic mutations are

ultimately responsible for the genesis of cancer and that there may be normal mechanisms that suppress cancer growth.

HORMONAL FACTORS IN CARCINOGENESIS

Hormonal exposure is related to about one-third of all new cancers. The incidence of breast cancer, prostate cancer, endometrial cancer, ovarian cancer, and perhaps testicular and other germ cell cancers has been linked to long-term exposure to various hormones.

Cumulative lifetime exposure to estrogen is the single greatest risk factor for development of breast cancer. Estrogen exposure, integrated over the lifetime of the patient, explains why risk is increased in women with an early menarche or late menopause, and in women who never bear children. Breast cancer risk is reduced 10 to 20% for each year menarche is delayed. Women who stop menstruating before age 45, because of either menopause or surgical intervention, have a 50% lower risk of breast cancer compared with women who menstruate beyond age 55. Obese women tend to have an increased risk of breast cancer after menopause, probably because adipose (fat) cells are capable of synthesizing estrogen, so that serum estrogen levels in obese women are higher than in nonobese women. Women with breast cancer have higher levels of serum estrogen than healthy control women, and estrogen levels tend to be higher in those populations of women with a high incidence of breast cancer. Although the mechanism of action of estrogen in causing breast cancer is unknown, breast cells proliferate more rapidly in response to estrogen stimulation.

Similarly, prostate cancer incidence is related to cumulative exposure to testosterone. In lab rats, injection of testosterone can induce prostatic adenocarcinoma. In humans, serum levels of testosterone in young black men are higher than in young white men, which may account for the twofold higher incidence of prostate cancer in black men later in life. The differences in risk in later life may be set at a relatively early age, since there is a clear difference in incidence rates even for black and white men in their 40s, the age at which prostate cancer first appears. The mechanism by which testosterone exposure causes an increased risk of prostate cancer is unknown, but testosterone levels directly control mitotic activity in the prostate.

Ovarian cancer incidence is related to ovulation in a complex way, but the basic mechanism appears to be hormonal in nature. Ovarian cancers develop

from epithelial cells on the surface of the ovary, whose growth is stimulated during ovulation. After each ovulation, the cells proliferate to cover the exposed portion of the ovary. Factors that prevent ovulation, including oral contraceptives, tend to have a protective effect against ovarian cancer. In women who have used oral contraceptives for 5 years or more, the relative risk of ovarian cancer is reduced about 40%, while the relative risk of endometrial cancer is reduced 55%, relative to women who have not used oral contraceptives.

As clear as these relationships between hormonal exposure and cancer are, it is not clear how that information should be used. It is neither practical nor desirable to recommend that people be given an ovariectomy or orchiectomy merely because surgery would decrease their risk of the associated cancer.

EXTRINSIC CAUSES OF CANCER

Most people are aware that there are carcinogens in the environment that can lead to a significantly increased risk of cancer. The known extrinsic carcinogens include chemicals, viruses, dietary factors, and radiation. But most people are unaware that perhaps two-thirds of all cancers are caused by extrinsic factors that can potentially be controlled or eliminated. To be specific, about 30% of all cancers are caused by tobacco use, about 15% are related to exposure to viruses, perhaps another 15% are related to dietary factors, and about 5% are related to occupational exposure to various carcinogens.¹²

Chemical Carcinogenesis

Chemical carcinogenesis is cancer causation that can be directly linked to exposure to a particular chemical encountered in the environment or on the job. Chemical carcinogenesis was born as a science in 1915, when several Japanese researchers reported that chronic application of coal tar to the ear of a rabbit could induce skin cancer. Many of the earliest studies of chemical carcinogenesis relied on repeatedly painting a suspected carcinogen onto rat or rabbit skin, and later examining that skin for tumor nodule production. This type of procedure serves as a biological assay for carcinogenicity. But this experimental approach often underestimates the carcinogenicity of a particular substance, because that substance may require metabolic activation within the body. Metabolic activation occurs when an inactive (or less active) form of a chemical

carcinogen is partially degraded by normal enzymes of the body to a form that is a more potent carcinogen.

There is now an extensive list of chemicals known to cause cancer in humans, including aflatoxin, arsenic, asbestos, benzene, chromium, coke oven emissions, diethylstilbestrol (DES), mustard gas, vinyl chloride, and various drugs used for cancer chemotherapy. Aflatoxin is a toxin produced by a mold that infects peanuts, and it is a very common carcinogen in parts of the world where peanuts form a major dietary element. But most other human carcinogens are encountered in significant amounts only by people whose occupation involves exposure, and occupational exposure to carcinogens probably accounts for less than 5% of all cancers. The number of cancers induced by most chemical carcinogens combined is a small fraction of the number of cancers induced by one carcinogen to which almost everyone is exposed, on a daily basis: tobacco smoke.

Carcinogens in tobacco smoke have been implicated in the causation of nearly one-third of all human cancers, including cancers of the oral cavity, esophagus, lung, kidney, bladder, and pancreas. Particulate matter in tobacco smoke, called tar, contains an array of chemicals known to be contact carcinogens. Among the carcinogens in tar are polycyclic hydrocarbons, those chemicals that induced tumors when applied to the ears of rabbits. Furthermore, metabolic activation of tobacco smoke components can produce carcinogens with the capacity to affect internal organs distant from the site of actual smoke exposure. Recent research has shown that there are even carcinogenic effects on the placenta in pregnant women who smoke. Banning the sale of tobacco would probably be a more effective anticancer measure than all of the environmental remediations proposed as a part of the Environmental Protection Act.

Chemical carcinogens have been shown to fall into two categories: those that can have a direct effect on cells and those that work indirectly. Direct-acting carcinogens have a chemical structure that permits them to interact with a target molecule, which is often DNA. Because of their inherent reactivity, most direct-acting carcinogens do not survive long enough in the environment to present much of a hazard to the general populace. Indirect-acting carcinogens are a far more insidious threat, because they are chemically stable and can exist in the environment for long periods of time as procarcinogens. These molecules are not, in themselves, carcinogenic, but they can undergo metabolic activation to become carcinogenic. Many factors, such as sex, age, diet, and genetic makeup, can influence the rate at which a procarcinogen is activated in a particular individual, so these factors can affect the carcinogenicity of a

procarcinogen. For example, male rats are more susceptible to liver cancer than female rats when given acetylaminofluorene. This is because male rats typically have higher levels of an enzyme that catalyzes activation of the procarcinogen.

Chemicals can potentially function as initiators, promoters, or cocarcinogens.¹³ An initiator is a carcinogenic chemical that can rapidly induce an irreversible change in the cell genome. This genetic change alone may lead to development of a tumor. A promoter is a chemical that can increase the response of a cell to a particular initiator if the cell is first exposed to the initiator. A promoter may not be carcinogenic or mutagenic in itself, but it can magnify the effect of an initiator even if applied long afterwards. This is potentially very important because it implies that a person could be exposed to an initiator at an early age, remain tumor-free for an extended period of time, then develop a tumor after exposure to an environmental promoter. A cocarcinogen is a chemical that is not carcinogenic by itself, but can enhance the action of a particular carcinogen when applied with that substance. An important example of this is alcohol used in conjunction with tobacco.

Chemical induction of an increased rate of cell division (mitogenesis) has been implicated in carcinogenesis and is conceptually similar to tumor promotion. But chemical stimulation of mitosis typically occurs only at very high levels of exposure to a chemical. Mammalian cells have very well-developed defenses against low levels of mutation, since DNA can be repaired very efficiently in normal cells. This may mean that the full carcinogenic effect of a chemical will be felt by cells only when cell mitosis is stimulated by high levels of that chemical. This argument implies that there is a threshold level of chemical exposure, below which no mutation will result. Testing the carcinogenicity of a drug at high levels of exposure therefore may not be informative about the carcinogenic potential of that chemical at low, chronic levels of exposure. It has been argued that the carcinogenicity of many chemicals has been overestimated by relying on tests in which animals are exposed to high levels of a chemical, since high levels of a chemical are expected to induce rapid cell division as well as genetic damage.⁹

Practically speaking, it is probably impossible to eliminate carcinogens from the environment. Risk analysis is now used to calculate the relative danger posed by a particular chemical, to determine whether extensive remediation efforts should be undertaken for that chemical. Risk analysis extrapolates from animal data, usually obtained at exposure to high levels of a potential carcinogen, to calculate the risk of human cancer from exposure to very low levels of

that chemical. Risk analysis thus assumes that there is a linear relationship between exposure and cancer risk, and that no threshold for carcinogenic effects exists. This assumption is probably not always valid, since several chemicals have been identified that have a threshold below which they are not tumorigenic. Generally, if the cancer death rate is elevated by less than 1 in a million, this is deemed to be an acceptable risk for society. This would seem reasonable, since the chances of dying in a car accident are very much higher.

Viral Carcinogenesis

Viruses were first identified as a potential cause of cancer in 1908, when Vilhelm Ellerman and Oluf Bang transmitted leukemia to chickens, using a fluid that had been filtered free of cells. Shortly afterwards, in 1911, Peyton Rous produced a solid tumor in chickens by injecting a cell-free extract of chicken sarcoma cells. But it was not until 1979 that Robert Gallo and his associates were able to demonstrate conclusively that a virus is the causative agent in human T-cell leukemia. Now it is thought that viruses may be the cause of up to 15% of all human cancers.

A virus is a tiny particle, much smaller than a bacterium, composed of a protein shell around a core of genetic material. That genetic material is usually composed of RNA, although some viruses have DNA instead. Viruses are incapable of replicating themselves unless they use the replicative apparatus of a cell. Viruses gain access to a cell, then usurp the metabolic machinery of the cell in order to make viral proteins and new copies of viral RNA. After the virus has replicated itself using the host cell machinery, dozens of new viral particles are released (Fig. 15). Each of these new viruses is capable of repeating the cycle, attacking new host cells and replicating itself. While this sort of attack does not usually result in the death or impairment of the host cell, the virus can still induce the host cell to make substantial quantities of viral protein and viral RNA. In fact, up to 1% of all the messenger RNA in an infected cell may be viral in origin, and expression of viral RNA can result in significant alterations of the form and function of the infected cell.

The mechanisms by which viruses cause human cancer are unclear, but there are a number of viral actions that might be involved in carcinogenesis. Viruses could potentially induce human cancers by: direct stimulation of cell proliferation through expression of a viral oncogene; induction of a host cell response such as inflammation or regeneration, accompanied by an indirect

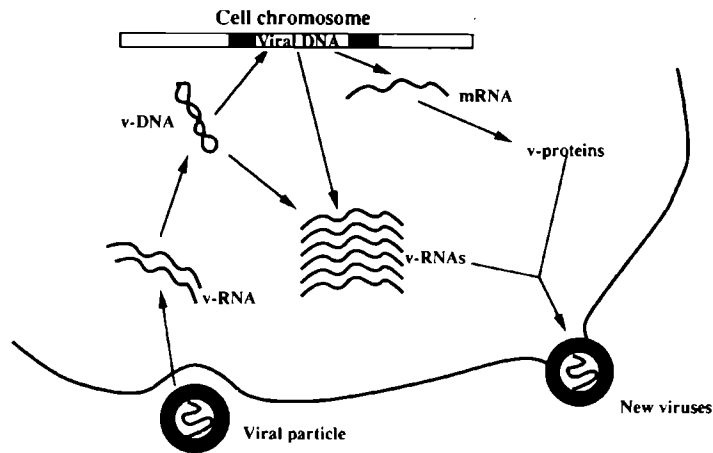


Figure 15. The life cycle of a virus. The viral particle is initially taken up into a cell because the virus binds to the cell membrane. Once the virus has bound to the cell, the viral genetic material, which in this case is RNA (v-RNA) is released into the cell cytoplasm where it rapidly synthesizes viral DNA (v-DNA) using the RNA as a template. The v-DNA can integrate into the chromosome of the cell (as a provirus) or it can simply begin to transcribe more v-RNA. In any case, new v-RNAs are combined with newly synthesized viral proteins (v-proteins) to make new viral particles. These new viral particles can be released from the cell membrane and infect nearby cells. This whole process can occur without necessarily harming the host cell. (From P. Neiman, 1987, *Harrison's Principles of Internal Medicine*, edited by E. Braunwald *et al.* Reprinted by permission of McGraw-Hill, Inc.)

stimulation of host cell proliferation; direct mutagenic effects on the host cell caused by viral integration into host DNA; suppression of the immune system of the host; or insertion of viral genetic material into the host DNA so that DNA transcription is induced. The latter could be particularly damaging to the host cell if DNA containing an oncogene was activated by the virus.

The first virus to be definitely linked to human cancer was the human T-cell leukemia-lymphoma virus, called HTLV-I. This virus is closely linked to a form of leukemia-lymphoma prevalent in coastal areas of Japan, in the Caribbean, and in regions of central Africa. The virus is transmitted by breast feeding, sexual intercourse, blood transfusions, and needle sharing. The latency period between infection and development of leukemia-lymphoma can be several decades, suggesting that viral exposure alone is not sufficient for development of disease. HTLV-I antibodies are present in more than 1 million people in Japan, showing that exposure to the virus is widespread, but only about 500 individuals a year develop the associated form of leukemia-

lymphoma. Laboratory studies have shown that this virus can immortalize certain human white blood cells, but the mechanism by which HTLV-I induces cancer is unknown.¹⁴

The hepatitis B virus (HBV) causes hepatitis, a chronic inflammation of the liver, and has also been linked to liver cancer. Areas of the world with high levels of chronic HBV infection, such as China, southeast Asia, and South Africa, tend to have a very high incidence of hepatocellular carcinoma. Results of a prospective study of the relationship between hepatocellular carcinoma and HBV were so strong that there is now a clinical trial under way, evaluating whether vaccination against HBV will protect a vulnerable population from liver cancer. Hepatocellular carcinoma usually does not appear until decades after a persistent HBV infection, and an interaction between HBV and a chemical carcinogen in food (aflatoxin) may be required for carcinogenesis. HBV appears to stimulate host liver cell proliferation both directly and indirectly, and it may also insert a viral transcription promoter into host cell DNA.

The Epstein–Barr virus (EBV) has been linked to several benign diseases of cell proliferation and to at least four different kinds of human cancer. EBV antibodies are found in about 90% of the population of the United States, and in virtually 100% of the population in other parts of the world. In the United States, EBV exposure often occurs in adolescence, when it causes infectious mononucleosis. EBV persists in the infected host throughout life, and virus shedding regularly occurs in saliva. Infection with EBV has been closely associated with Burkitt's lymphoma and cancer of the nose and mouth, and may cause Hodgkin's lymphoma and B-cell lymphoma as well. The link between EBV and B-cell lymphoma is clearest in AIDS patients, and in transplant patients who are immunosuppressed to prevent organ rejection. EBV infection of B cells in culture leads to rapid cell transformation and immortalization, although the transformed B cells will not induce a tumor even in mice with a defective immune system. Thus, it is unclear how EBV infection leads to carcinogenesis.

Human papillomavirus (HPV) has been implicated as a causative agent in cervical and other genital cancers, as well as cancers of the anal region, with exposure to the virus probably occurring during sexual activity. Together these cancers make up about 10% of the cancers occurring worldwide. Viral DNA is found in about 90% of all cervical cancers, and usually the viral DNA is integrated into the DNA of the human cells. HPV can immortalize certain cultured human skin cells, and some of the immortalized cells become capable

of causing a tumor if grown in culture for a long period of time. As with the other cancer viruses, infection with HPV alone is not sufficient for development of cancer; additional modifications of host DNA appear to be required in a stepwise progression to malignancy.

An association has been noted between the virus that causes AIDs and several ordinarily rare cancers. AIDS is caused by the human immunodeficiency virus (HIV), whose primary action against the patient is suppression of the immune system. However, this immunosuppression is associated with an increased incidence of Kaposi's sarcoma and B-cell lymphoma in AIDS patients. Recent evidence suggests that Kaposi's sarcoma, a rare malignancy of the cells that form blood vessels, is actually caused by cytomegalovirus (CMV), but that chronic CMV infection usually only occurs when the patient is already immunosuppressed by HIV.

Dietary Factors in Carcinogenesis

There is a developing consensus that the major cancers of the gastrointestinal tract, including stomach, colon, and rectal cancer, are causally related to certain dietary factors. These cancers accounted for about 17% of new cancers in the United States in 1990, so dietary factors in carcinogenesis may be far more important than was previously recognized.

Epidemiological studies of cancer prevalence in groups of people with different diets have shown a link between consumption of animal fat and the incidence of colorectal cancer. An association between colorectal cancer risk and consumption of fat from dairy products is also significant, but is weaker than the association with animal fat from red meat. In rodents, consumption of a diet high in saturated fat induces inflammation of the bowel, with a loss of cells from the lining of the colon. This results in a compensatory increase in the growth rate of the remaining cells, in order to regenerate the normal lining of the colon. This stimulatory effect of saturated fat on cells of the colon has been implicated as the mechanism of dietary carcinogenesis. It has been postulated that a 50% reduction in the consumption of animal fat might result in a similar decrease in the incidence of colorectal cancer, although this is very controversial.

High dietary intake of fat has also been associated with an increased risk of cancers of the breast, ovary, endometrium, and prostate. Data in support of these claims are sparse, but the Canadian National Breast Screening Study did

show an elevation of breast cancer risk in those women with the highest level of dietary fat consumption. But this association was weak, and there was no evidence of a dose-response relation between fat consumption and breast cancer incidence. In addition, several other studies have failed to find any association between fat consumption and breast cancer. Those few studies of the association between dietary intake of polyunsaturated fats and breast cancer incidence are even more inconclusive.

Considerable evidence now shows that a diet low in crude fiber is associated with an increased risk of colon cancer. In the Nurses Health study, women with the highest animal fat intake and the lowest dietary fiber intake had the highest risk of colon cancer. Dietary fiber consists of those parts of a food plant that are resistant to digestion in the human intestinal tract. A high intake of dietary fiber generally causes food to move more rapidly from the stomach through the intestines, resulting in a shorter transit time through the gut. Dietary fiber also tends to increase the water content of material in the intestines. Thus, fiber causes dietary carcinogens to move more rapidly through the gut and to remain in a more dilute state as they move. Several governmental agencies recently suggested that Americans should double their daily intake of dietary fiber to about 20 to 30 grams a day.

In the period between 1973 and 1987, the incidence of stomach cancer declined by 20.5%, while the mortality from this disease declined by 29.4%. This impressive reduction in stomach cancer incidence and mortality is associated with a decline in the use of salting and pickling to preserve food and an increase in the average consumption of fresh fruits and vegetables. Mortality rates from stomach cancer correlate very well with measures of dietary intake of salt, and this correlation holds between different cultures. A direct relationship between the consumption of salty foods and the incidence of stomach cancer has even been observed in several different prospective studies. High salt intake has a corrosive effect on the cells lining the stomach, leading to cell injury and death, with regeneration of the stomach lining by surviving cells. Therefore, salt intake may be carcinogenic through the familiar mechanism of stimulated cell proliferation.

Total dietary intake of calories appears to be an important risk factor for certain cancers. If caloric intake greatly exceeds caloric expenditure, then obesity results. Obese women have a higher postmenopausal incidence of breast cancer than lean women, and numerous studies have shown that countries with high dietary fat consumption have an increased incidence of breast cancer. High caloric intake in childhood causes early puberty, so that the

cumulative lifetime exposure to estrogen is greater. Conversion of a precursor molecule into estrogen may actually occur in adipose tissue, so an increase in body fat could result in production of more estrogen. Obesity has also been implicated as a causative factor for endometrial cancer.

Alcohol consumption has been associated with cancers of the mouth, pharynx, larynx, esophagus, colon, rectum, liver, and breast. Alcohol also has a strong interaction with tobacco use in the causation of lung cancer. Alcohol stimulates proliferation of rectal cells in the rat, so a causative interaction is at least plausible between alcohol consumption and colorectal cancer. But no clear mechanism has been postulated for the association between alcohol intake and breast cancer incidence.

The diet can also provide access to micronutrients that appear to protect the individual from cancer.¹² A consistent but moderate decrease in lung cancer incidence has been noted among persons with a high dietary intake of β -carotene, a micronutrient present in many vegetables. There is some evidence suggesting that β -carotene may protect against other types of epithelial cancer as well. Prospective studies have found that patients who develop cancer, especially lung cancer and other smoking-related cancers, are likely to have lower serum levels of β -carotene than healthy controls. Furthermore, smokers generally consume less β -carotene than nonsmokers, so the evidence is fairly strong that β -carotene can reduce cancer incidence. β -Carotene is a member of a class of compounds that can act as antioxidants, quenching the oxidizing activity of certain potent carcinogens such as singlet oxygen. There is weak but suggestive evidence that certain other antioxidants, including vitamin C and vitamin E, may also protect against cancer.

Radiation Carcinogenesis

Radiation is probably the most widely recognized and intensively studied of all human carcinogens. This is, in large part, the result of fears aroused by the Cold War and the Japanese experience with atom bomb survivors after the Second World War. The disaster at Chernobyl heightened anxiety about radiation exposure through accidental release of radioactive waste from nuclear reactors. Yet about 80% of all human exposure to man-made sources of radiation has come from medical X-rays.

Radiation is harmful because it interacts with electrons in the atoms of any material exposed to radiation. If the radiation is energetic enough, it will cause electrons to be ejected from those atoms in a process called ionization. The ejected electrons then interact with other atoms to produce highly reactive ions called free radicals. Alternatively, radiation may interact directly with the atomic nucleus, so that protons or other charged particles are created within the material exposed to radiation. In either case, damage to biological tissue is actually mediated by the ionized particles created by radiation, so harmful radiation is often referred to as ionizing radiation.

The clearest data available on the relationship between radiation exposure and cancer induction in humans have come from the thorough follow-up of Japanese survivors of the atom bomb blasts. These data have been used to calculate the increased risk of dying from various cancers for persons exposed to 2 Gray or more radiation, as compared with unexposed persons. A Gray is a unit used to describe the amount of energy absorbed per unit volume of tissue. For comparison, the total exposure to radiation occurring during radiotherapy is typically in the range of 30 to 60 Gray, with radiation often fractionated into many small exposures of 1.5 to 2.5 Gray each. But a major difference exists between radiation exposure during radiotherapy and the radiation exposure received by atom bomb survivors, in that radiotherapy involves exposure of a very small portion of the body to radioactivity, while the bomb survivors usually received whole-body exposures.

The most striking effect of whole-body radiation exposure is an increased mortality from leukemia. The relative risk of dying from leukemia was increased nearly 15-fold among bomb survivors exposed to 2 Gray or more radiation. Relative mortality from all other cancers combined, or from cancers at many specific sites, was elevated only about 2-fold with respect to an unirradiated group of people. Mortality from multiple myeloma was about 5-fold higher than normal, but mortality from cancers of the pancreas, rectum, and uterus was not elevated at all. However, these estimates of increased mortality from radiation exposure are subject to a certain amount of error. Estimates are based on a 28-year follow-up of the atom bomb survivors, over the period from 1950 until 1978. If certain cancers that were induced by radiation take longer than 33 years to develop, then incidence of these cancers would be underestimated in this study.

The most generally accepted criterion for determining whether any particular agent causes cancer is determining whether there is a dose-response

relationship between exposure and cancer incidence. Thus, if radiation causes cancer, one would expect persons exposed to high levels of radiation to have a higher incidence of the cancers caused by radiation. This dose–response relationship has been clearly shown for leukemia in the Japanese bomb survivors, for all cancers combined (excluding leukemia), and for cancers of the breast.

Dose–response relationships between radiation exposure and cancer induction have also been shown in certain groups of people exposed to radiation for medical purposes. Some of these studies are of particular interest because they involve exposure of small tissue volumes to radiation, or exposure of tissue to radiation that was fractionated into many, small exposures. For example, postpartum mastitis, or inflammation of the lactating breast, was often treated with radiation in the past. It is now known that this exposure led to an increased incidence of breast cancer in many of the irradiated patients. Similarly, radiation treatment of acne led to an increased incidence of thyroid cancer, and radiation treatment of ankylosing spondylitis, a chronic inflammatory disease of the spine, led to an increased incidence of leukemia, lung cancer, and cancers of the spinal cord.

A thorough study of the dose–response relationship between childhood X-ray exposure and the incidence of thyroid cancer has pinpointed a weakness in our understanding of carcinogenesis. When cancer incidence is plotted against the cumulative dose of radiation to the thyroid, a clear dose–response relationship is seen. However, the data available are for radiation exposures of 1 Gray up to about 7 Gray. These data are open to a number of different interpretations if the question becomes, what happens to patients exposed to very low doses of radiation? While the dose–response relationship is clear-cut at higher doses, there are little or no data available for exposures of less than 0.1 Gray. The existing data can be fitted reasonably well with a straight line drawn through the data points. This linear relationship would imply that risk of cancer increases linearly with exposure, and that there is no threshold of exposure below which radiation is not harmful. Alternatively, the data points can be fitted with a curved line, which actually seems to provide a better fit. But this would imply that high levels of exposure to radiation are very harmful, while low levels of exposure could fall below a threshold, so that there might be no risk from very low levels of exposure. The question of whether or not such a threshold exists is a nagging issue, not only in radiation carcinogenesis, but in all types of carcinogen exposure.

Sunlight Carcinogenesis

Certain wavelengths of light, particularly in the ultraviolet (UV) range, are known to be carcinogenic, and sunlight is the major causative agent for skin cancer. UV light is considered to be a nonionizing radiation because it affects molecules differently than does ionizing radiation. UV light induces molecular motion, rather than the molecular ionization induced by radiation exposure. Of the three types of UV light in sunlight, UV-B has been most closely linked to skin cancer. UV-A light is less damaging, and UV-C light, while very dangerous, generally is absorbed by the atmosphere of the Earth. UV-B light is present in the lights used in tanning salons, so this light can potentially be carcinogenic. Other types of nonionizing radiation that might conceivably be carcinogenic are: visible or infrared light; ultrasound; microwave radiation; radio frequency radiation; and the electrical fields associated with high-tension power lines.

In general, nonionizing radiation is far less energetic than ionizing radiation. That is why exposure to sunlight induces primary cancers in the skin only, while exposure to ionizing radiation can induce primary cancers in tissues deeper inside the body. A clear dose–response relationship has been established between sunlight exposure and skin cancer incidence.

MULTISTAGE CARCINOGENESIS

The process of carcinogenesis is complex and poorly understood, whether the carcinogenic agent is a chemical, a virus, or radiation. But a better understanding of these complexities has been achieved over the last 50 years, through the use of mice as an experimental model of skin cancer. In a typical mouse skin carcinogenesis experiment, an experimental group of mice might be exposed to a chemical initiator, while a control group would not be exposed to the initiator. Then both experimental and control animals might have a tumor promoter painted onto their skin for several weeks. If the initiator–promoter combination does enhance skin carcinogenesis, then the experimental group of animals would be expected to develop multiple tumors in the area where the promoter was applied, while the control animals should have few or no tumors.¹⁵

Exactly this experiment has been done with hundreds of combinations of initiators and promoters, and has led to a general acceptance of the idea that carcinogenesis is a multistage process. An initiating agent can be any carcinogenic agent that induces some irreversible genetic change. Exposure to an initiating carcinogen could potentially involve a very low level exposure, so that a cancer might not be produced in the normal lifetime of a person. But that person is nevertheless primed for later exposure to a promoter. A tumor promoter could be any agent that enhances proliferation of the initiated (mutated) population of cells. Exposure to the promoter could result in formation of precancerous lesions, each of which could then progress to form a malignant tumor. Numerous experiments have shown that if the promoter is given before the initiator, no tumors develop. This suggests that carcinogenesis is a multistage process that occurs only if the stages happen in the correct order. However, many environmental carcinogens are complete carcinogens, meaning that they are capable of both initiating and promoting a tumor.

A multistage model of carcinogenesis has been invoked to explain how retinoblastoma develops in certain patients. Retinoblastoma (RB) is recessive, and tumors develop only when both normal copies of the RB gene are lost (Fig. 14). Patients with RB typically have one normal RB gene and one mutant RB gene in most of their cells. Since one normal RB gene is generally sufficient for tumor suppression, a tumor could develop only if the normal RB gene is lost. But most individuals carrying one mutant RB gene develop multiple RB tumors because loss or inactivation of the normal RB gene appears to be relatively common. Apparently the mutant RB gene is duplicated and replaces the normal RB gene during cell mitosis, so that the cell winds up with two identical mutant RB genes. A chromosomal event in which the mutant RB gene replaces the normal RB gene may affect as many as one in 10,000, or even one in 1000 cells every cell generation. This explains why RB tumor cells typically have two identical mutant RB genes, while the rest of the cells of the body often retain one normal RB gene. In a sense, individuals with only one normal RB gene are “initiated,” and if this normal gene is lost through mutation, then tumor formation is “promoted.” Multistage carcinogenesis is therefore analogous to a “two-hit model” of tumor development: two genetic accidents are required for tumor development, with the first of these accidents being an inherited absence of one normal RB gene. When the remaining normal RB gene is lost, this constitutes the second “hit” and a tumor is formed (Fig. 16).

The idea that carcinogenesis is a multistage process has recently received strong support from work on multistage carcinogenesis in colorectal cancer. In

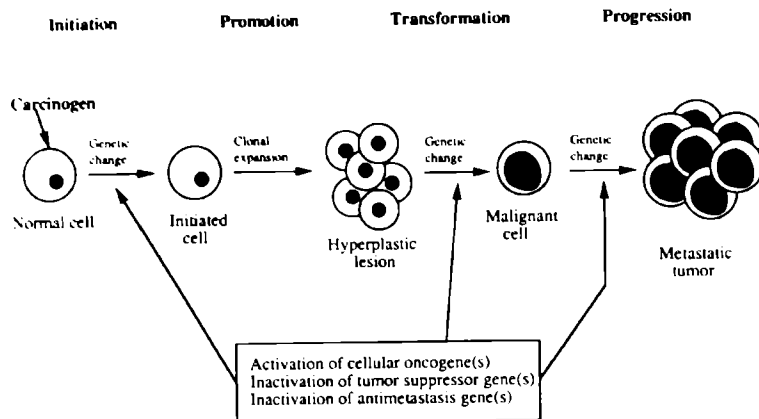


Figure 16. Carcinogenesis as a multistage process. A carcinogen is shown inducing a genetic change in a normal cell, which initiates that cell and makes it vulnerable to other genetic changes which combine to transform the cell to a malignant form. The genetic changes that cause initiation or promotion could involve mutations that activate a cellular oncogene, inactivate a tumor suppressor gene, or inactivate an antimetastasis gene. It should be noted that cells in the hyperplastic lesion, although they have suffered one genetic change, are not yet malignant. Tumor progression may be associated with more genetic changes than are required to generate a single malignant cell. (From C. C. Harris, 1991, *Cancer Research* 51:5026s. Reprinted by permission of the American Association for Cancer Research.)

this cancer, the initiating event was a mutation that caused loss of part of one chromosome, while the promoting events were additional mutations affecting the DNA. An analysis of the DNA obtained from colorectal tumors of 50 women showed that each of these tumors probably arose as a clone of a single transformed cell.^{16,17} Evidence for the clonal origin of these colorectal tumors came from an analysis of the pattern of X-chromosome inactivation in tumor cells. Basically, since all women have two X chromosomes, yet only one is needed for the cell to survive, each cell of a woman's body randomly inactivates one of the two X chromosomes. Because inactivation is random, half of the cells inactivate one X chromosome, while half of the cells inactivate the other. Yet, in each of the colorectal tumors, all of the tumor cells inactivated the same X chromosome, suggesting that these cells were originally derived from a single transformed cell.

Examination of colorectal tumor histology showed that 30 of 50 tumors were adenomas, a benign cancer, while 20 were malignant carcinomas. Genetic analysis showed that a loss of one region of chromosome 17 had occurred in 75% of the carcinomas, but had occurred in only 3% of the adenomas. In two

tumors that contained both adenoma and carcinoma cells, only the carcinoma cells lost the relevant part of chromosome 17. This suggests that a gene on chromosome 17 suppresses tumor growth and that loss of this gene is associated with progression from the benign to the malignant state. The gene on chromosome 17 has now been identified as a tumor suppressor gene called p53. Many of the more malignant colorectal tumors showed substantial losses of genetic information in addition to loss of p53. In fact, there was an average loss of 20% of the genetic information from chromosomes of individual tumor cells, and some tumors were composed of cells that had lost a third to a half of all genetic information. Patients with tumors with an unusually large loss of genetic information tended to have a poorer prognosis than normal. It has been proposed that numerous tumor suppressor genes are scattered throughout the genome and that inactivation or loss of these genes has a detrimental effect on the regulation of cell growth. Thus, malignant tumors are viewed as having progressed further than benign tumors in terms of a stepwise loss of genetic information.¹⁸

This concept of multistage carcinogenesis seems quite different from the concept of initiation and promotion of a tumor, but these differences are smaller than they might appear. In a broader perspective, an initiating event would be any event that induces some irreversible genetic mutation, including loss of a tumor suppressor gene such as p53. A promoting event would be any event, including loss of additional genetic information, that enhances proliferation of the mutated cells. Progression from a benign to a malignant state could be associated with either stepwise loss of genetic information or stepwise promotion of the rate of cellular proliferation. This new concept of multistage carcinogenesis even allows complex interactions between the genome and the environment to induce tumor growth. Carcinogenesis is thus possible through unfortunate confluences of unrelated events, such as a random mutational event acting as an initiator, followed by exposure to a chemical promoter, or an initiation event induced by exposure to radioactivity, followed by random mutational loss of tumor suppressor genes.

CHAPTER 4

Oncogenes and Tumor Suppressor Genes

When tumor cells divide, they transmit cancerous traits to their daughter cells. For this reason, it has generally been assumed that inheritance of these cancerous traits is determined by specific genes. The specific genes associated with transformation of a normal cell to a malignant cell have been called oncogenes. Scientific interest in oncogenes dates back to 1911, when Peyton Rous showed that sarcomas could be induced to grow in chickens by injection of a cell extract prepared from a chicken sarcoma. This extract induced sarcomas at the point of injection, even though the extract was filtered free of cells, showing that the cancer-inducing agent in the extract had to be smaller than a cell. We now know this agent to be a virus.

This was the first clue that viral genes could be involved in cancer formation, and it led to a great deal of work on viral carcinogenesis. But the idea that a cell could be made malignant by a transmissible agent that is largely composed of genes had an impact far beyond the field of viral carcinogenesis. People began to investigate the relationship between specific gene mutations and cancer, and this research has stimulated many of the advances made in molecular biology over the last two decades. In general, the fact that a particular gene is associated with a particular type of cancer is the clearest possible demonstration that cancer results from a genetic change in cells.

EVIDENCE FOR A GENETIC CAUSE OF CANCER

One of the strongest pieces of evidence for a genetic cause of cancer is the existence of several hereditary syndromes in which there is a dramatic familial increase in cancer incidence. One of the most striking of these familial cancer syndromes is Li–Fraumeni syndrome, in which there is a pattern of increased susceptibility to soft tissue sarcomas, leukemia, and cancers of the breast,

brain, bone, and adrenal glands. Patients with this syndrome tend to contract cancer as children or young adults, and the cancer often occurs at multiple sites. A prospective study of 545 members of 24 families with Li–Fraumeni syndrome showed that cancer risk below age 20 was 21-fold higher than in the normal population. Of those family members under age 45 who developed cancer, at least 87% of the cancers were associated with the specific tumor susceptibility gene carried by these families.¹⁹

Another strong piece of evidence for a genetic cause of cancer is the frequency with which abnormal chromosomes are detected in tumor cells. The best known example of this is the “Philadelphia chromosome” (Fig. 2), frequently seen in patients with chronic myelogenous leukemia (CML). More than 95% of patients with CML have the Philadelphia chromosome, a very strong argument that chromosomal aberrations can cause cancer. Chromosomal changes that affect a single gene can ultimately result in production of a tumor, as shown by recent work on colorectal cancer. About 70% of human colorectal cancers show deletion of a small part of chromosome 18, and a putative tumor suppressor gene has been identified on the deleted segment of this chromosome. This tumor suppressor gene, called DCC (for deleted in colorectal carcinoma), codes for a protein that may be involved in the adhesion of cells to one another. The DCC gene is expressed in most normal cells, including colon cells, but its expression is sharply reduced or absent in colorectal tumor cells. Alteration of the DCC gene may lead to a loss of normal cellular growth regulation, by altering interactions between the tumor cell and the cells surrounding it, or between the tumor cell and the extracellular matrix.

Another example of a chromosomal change that affects a single gene, but which can result in production of a tumor, is seen in glioblastoma multiforme, a type of brain tumor. Analysis of glioblastoma cells excised during brain tumor surgery shows that 40–50% of the samples have an amplified gene for the epidermal growth factor receptor (EGFR) protein.²⁰ Similarly, more than 90% of human medulloblastoma cells grown in culture show amplification of either the EGFR gene or another gene called *N-myc*. Such genetic alterations provide a growth advantage to these cells in culture and may also be involved in tumor growth in the patient.

That genetic mutations can cause cancer is clearly shown by certain diseases in which there is an impaired ability of cells to repair damaged DNA. One of the best-studied conditions in which gene repair is deficient is xeroderma pigmentosum (XP). Patients with this recessive genetic trait have incidences of skin cancer, including epidermal carcinoma and malignant

melanoma, that are several thousandfold higher than the general population. Epidemiological evidence shows that these skin cancers are induced even in normal patients by exposure to the ultraviolet (UV) portion of sunlight. But patients with XP are deficient in their ability to repair DNA damage caused by UV exposure, so they contract cancer at a much higher rate than normal persons. Interestingly, XP patients are also more likely to contract cancers of the oral cavity, which may be caused by chemical carcinogens in the diet.

Additional evidence supporting the hypothesis that genetic mutations cause cancer is seen in the relationship between mutagenicity and carcinogenicity. Mutagenicity is the ability of a chemical to induce DNA mutations, while carcinogenicity is the ability of a chemical to induce cancer. Most carcinogens are also mutagens, and the extent of DNA mutation induced by a chemical is usually directly correlated with the carcinogenic potential of that chemical. The relationship between mutagenicity and carcinogenicity has been used for years to rapidly assess the carcinogenic potential of a particular chemical. One test of chemical mutagenicity can predict which chemicals are carcinogenic with 90% accuracy, which confirms that most mutagens are also carcinogens.

THE NATURE OF ONCOGENES

It was originally thought that oncogenes were viral genes, and that normal cells were transformed to malignant cells under the influence of a virus bearing an oncogene. It is now known that this is not the case: while viruses can carry oncogenes, uninfected normal cells possess genes that are remarkably similar to viral oncogenes. The fact that the viral gene and the cellular gene are so similar implies that both genes have a common origin. It is now believed that viruses acquired oncogenes by accidentally picking them out of cells they had infected. In the confusion of an infected cell, a cellular oncogene is carried along by the virus when the viral DNA is packaged into new viral particles. Because cellular genes are thought to be the source of viral oncogenes, the cellular genes have been called proto-oncogenes. But, for the sake of simplicity, we will call the cellular genes oncogenes as well, and the origin of the oncogene will be explicitly stated (e.g., cellular oncogene or viral oncogene).

Oncogenes are usually given complicated names that reveal something about the history or biology of that gene in an obscure kind of code. For example, *v-myc* is a viral gene from the avian *myelocytomatosis* virus, which

induces leukemia in chickens. Another gene, called *c-myc*, is the normal cellular counterpart to *v-myc* and is found in a wide range of normal mammalian cells. Similarly, *v-Ha-ras1* is a viral gene from the *Harvey* sarcoma virus that induces sarcomas in rats. The normal cellular counterpart of this viral gene is called *c-ras^H* and is found on human chromosome 11. The fact that viruses can carry oncogenes into the cells they infect can have dramatic consequences, since expression of the oncogene is controlled by the virus, not the cell. Whether the protein product made from a viral oncogene is normal or abnormal, that protein can have a strong transforming effect on the cell.

There is a remarkable similarity in the structure of cellular oncogenes, whether those oncogenes are taken from a fungus, a bird, or an animal. For example, an antibody to the protein product of the rat *myc* gene can be used to show *myc* gene expression in yeast cells or in human cells. This striking degree of similarity between *myc* genes from very different organisms argues that the *myc* gene is serving a critical purpose in all of these cells. It is now thought that cellular oncogenes are required for cell growth and development. The human *myc* gene codes for a protein that binds to DNA and is required for DNA synthesis, so it has been concluded that this protein is involved in regulation of the cell cycle. Another cellular oncogene, called *c-sis*, codes for a protein that is very similar to a growth factor called platelet-derived growth factor (PDGF). Yet another cellular oncogene, called *c-erbB*, codes for the epidermal growth factor (EGF) receptor protein; as we will see, expression of *c-erbB* is amplified in some breast tumors.

Cells usually have a series of related oncogenes that together form what are called oncogene families. An example of this is the *ras* family of cellular oncogenes, five forms of which appear in human cells. Each gene codes for a protein that appears to be involved in the cellular response to a different growth factor, and mutation at a single point in any of the *ras* proteins can lead to cell transformation. If a human cell is transfected with a mutant form of one of the *ras* genes, that cell responds aberrantly to growth factors, implying that normal *ras* genes are required if cells are to respond normally to growth factors. Another example of an oncogene family is *myc*, three forms of which appear in human cells. All of the *myc* proteins bind DNA, although their specific function is unknown. More generally, it is also unknown why cells need to have whole families of related oncogenes functioning during cellular development. However, it is truly striking that oncogene families are so similar in various organisms, including such radically different organisms as yeast and humans.

EVIDENCE LINKING CELLULAR ONCOGENES TO TUMOR FORMATION

Cellular oncogenes appear to be a diverse collection of genes that are critical for regulation of normal cell growth and differentiation. Many of the cellular oncogenes code for proteins that are either growth factors or growth factor receptors, while other oncogenes code for proteins that seem to directly control progress through the cell cycle. Altered function of these oncogenes could thus have extremely deleterious effects on a normal cell, whether oncogene function is disrupted by viral infection or by some other means. Mechanisms by which oncogene function could be altered include: insertion of a viral oncogene into the cellular DNA; mutation of a cellular oncogene; translocation of a piece of a chromosome bearing an oncogene to another location, where that oncogene is regulated differently; or amplification of a chromosome bearing an oncogene.

Certain cells grown in culture have the capacity to incorporate exogenous DNA into their own cellular DNA, so that gene transfection (gene transfer) can be studied experimentally in cell culture. One cell line, called 3T3, can be induced to incorporate DNA if the DNA is simply precipitated onto the surface of the cells. These cells take up DNA coating their surface, transport this DNA into the cell nucleus, and incorporate it into their own cellular DNA. This sort of gene transfection may affect only one cell in 100,000, but this is sufficient since the transfected cell can be induced to grow and make progeny. Transfection of 3T3 cells with either an oncogene or the total chromosomal DNA of a transformed cell can result in transformation of the 3T3 cells to a cancerous form. Using this 3T3 cell assay for gene transfection, activated oncogenes can be detected in the DNA of many human tumors, even in tumors that are not induced by viruses. For example, if 3T3 cells are transfected with DNA from cancers of the lung, colon, bladder, pancreas, or ovary, the cells can be transformed. Transformation of 3T3 cells appears to be associated with transfection of an activated *c-ras^K* cellular oncogene. In fact, roughly 10% of all human cancers are associated with an activated *c-ras^K* cellular oncogene that can induce transformation of 3T3 cells. This shows that oncogenes are frequently activated in cancer cells, but the question remains whether oncogene activation is a cause or a consequence of cancer.

Recent experiments with the 3T3 cell line suggest that oncogene activation can cause cancer. If 3T3 cells are transfected with DNA from certain lung or

mammary carcinomas, the cells are transformed by an activated *c-ras^H* cellular oncogene. This oncogene codes for a protein called p21, which is usually expressed at high levels in cells transformed by the *c-ras^H* oncogene. If the *c-ras^H* oncogene is linked to a piece of viral DNA containing a promoter, the oncogene and promoter alone are able to induce cellular transformation. The ability of the *c-ras^H* oncogene to transform cells is usually associated with a single mutation in the gene sequence, so either altered expression or altered function of p21 may be sufficient to cause cancer.

Evidence is accumulating that altered oncogene expression can result in human tumor development. Translocation of the *c-myc* gene to a new chromosomal location is seen in some B-cell lymphomas. Translocation apparently results in the gene being differently regulated, since translocation is associated with oncogene activation in several different cancers. In B-cell lymphoma, translocation of the *c-myc* gene brings it into a position adjacent to genes involved in immune system regulation.²⁰ The *myc* family of oncogenes is not the only oncogene family involved in human tumor formation. In the *ras* gene family, mutation of *c-ras^{H-1}* is associated with colon carcinoma, amplification of *c-ras^{H-2}* is seen in embryonal carcinoma, and several acute leukemias are associated with mutation of *c-N-ras*.

It was estimated several years ago, based on relatively insensitive techniques, that activated cellular oncogenes are associated with a particular type of cancer in only about 10–15% of cases. If the association between an activated oncogene and a particular cancer is relatively rare, it may mean that oncogene activation is not causative of cancer, but merely occurs during tumor development. But recent application of more sensitive techniques has shown that up to 41% of human colorectal cancers are associated with *ras* gene mutations. Thus, it is possible that oncogene activation is causative of cancer.

Increased expression of certain oncogenes is also associated with progression of certain malignancies. For example, amplification of an *myc* gene was seen in 8% of untreated SCLC patients (3 of 40) and 28% of treated patients (19 of 67). This difference is significant and implies that oncogene expression is involved in tumor progression.²¹ The extent of amplification of *c-N-myc* in untreated neuroblastomas appears to correlate with the rapidity of tumor progression, and amplification predicts poor patient prognosis. In breast cancer, the greater the amplification of *c-erbB*, the shorter is the interval to tumor relapse. In colon carcinoma, the *c-ras^{H-1}* gene was amplified in a tumor that was identified as malignant carcinoma, but the gene was not amplified in a tumor identified as benign adenoma. An acute B-cell leukemia cell line was found to

have two different oncogenes activated simultaneously: one of the oncogenes was found in low-grade lymphoma, while the other oncogene was associated with Burkitt's lymphoma, a fairly aggressive tumor. When both oncogenes were activated together, the resulting cancer was very aggressive.

A very important experiment has shown that tumor production may require multiple changes in oncogene expression. Human umbilical cord blood contains B cells that can be made immortal by infection with Epstein-Barr virus (EBV). Although EBV infection is known to be involved in Burkitt's lymphoma, the immortalized B cells are not capable of inducing tumors in immunosuppressed mice. But if the *c-myc* gene is transfected into these immortalized B cells, the cells become capable of inducing tumors in mice and these tumors resemble Burkitt's lymphoma. Thus, neither EBV nor the *c-myc* gene alone is capable of producing a tumor, but they can cooperate to produce an aggressive tumor. Similarly, if *c-myc* and *c-ras* are transfected together into a single fibroblast cell, the oncogenes interact to produce a fully transformed cell capable of inducing an aggressive tumor. This experiment indicates that multiple genetic events are probably required for tumor development, consistent with our concept of multistage tumorigenesis.

GENETICS OF SPECIFIC HUMAN CANCERS

If oncogene activation is involved in cancer formation, then careful analysis of specific human cancers may show consistent involvement of a specific oncogene. The Philadelphia chromosome, a reciprocal translocation seen in 95% of chronic myelogenous leukemia (CML) patients, is an example of this (Fig. 2). Such a reciprocal translocation might seem unimportant since neither chromosome is actually lost and both daughter cells still have the same total genetic information as the mother cell. But the reciprocal translocation in the Philadelphia chromosome brings two genes together that are ordinarily on separate chromosomes. The cellular oncogene *c-abl* is located near the break point on chromosome 9, and this oncogene is translocated next to a gene on chromosome 22 called the break-point cluster region, or *bcr*. For some reason, this results in a quantitative and qualitative alteration in expression of the *c-abl* protein. The protein made from the mutated *c-abl* gene is larger than a normal *c-abl* protein, suggesting that it is a fusion of the proteins made from *c-abl* and *bcr*. It is unknown why this fused protein product results in development of CML.

Analysis of gene expression in human breast cancer has shown that about 30% of 189 patients with breast cancer showed amplification of an oncogene called HER-2/*neu*.²² This oncogene is a member of the *c-erbB* oncogene family, and it is similar to the EGF receptor. Oncogene amplification in breast cancer cells was detected by sophisticated molecular biological techniques that permitted researchers to measure the extent of oncogene amplification. In a subset of patients with positive lymph nodes, indicating a poorer prognosis, the HER-2/*neu* oncogene was amplified in 40% of patients. The degree of oncogene expression was closely correlated with the number of positive lymph nodes, suggesting that expression of this oncogene actually determines metastatic ability of the tumor. Amplification of HER-2/*neu* was associated with a shorter period of disease-free survival, and was a better predictor of survival than was hormone receptor status, age of the patient, or size of the primary tumor. Women with Stage I (node-negative) breast cancer are generally expected to have a good survival rate, but about 25 to 30% of these cancers recur nonetheless. Traditional methods of breast cancer staging are not adequate to identify which Stage I breast cancers are likely to recur. A preliminary study has shown that about 15% of women with Stage I breast cancer had HER-2/*neu* amplification. The clinical implications of this could be important, since HER-2/*neu* amplification may identify a subset of Stage I patients at higher risk of relapse. If oncogene amplification proves successful in identifying high-risk patients, these patients could be given more aggressive therapy.²²

Neuroblastoma is a nerve cell cancer of childhood, which frequently metastasizes to the liver, lungs, brain, bones, lymph nodes, and adrenal glands. It is commonly associated with abnormalities of chromosome number, as 44% of neuroblastoma cells are aneuploid. Neuroblastoma is also frequently associated with amplification of the *N-myc* oncogene, and amplification of this oncogene correlates well with disease stage and with overall prognosis. Amplification of *N-myc* is a better predictor of survival than simple tumor grading, since Stage II patients with oncogene amplification fare no better than Stage III patients, while Stage II patients without oncogene amplification are more likely to have a favorable prognosis. When neuroblastoma cells are grown in culture, they have an undifferentiated, primitive appearance. But about 10 to 20% of these cells spontaneously differentiate to form cells with the mature appearance of a neuron. When the concentration of serum in the growth medium is lowered, or when cells are treated with certain drugs, about 70 to 80% of cultured neuroblastoma cells can be induced to differentiate. Concurrent with this differentiation, cells begin to behave more like neurons, and expression of

the N-*myc* oncogene decreases. These findings are consistent with the observation that neuroblastomas can spontaneously regress in some children. The N-*myc* cellular oncogene may be directly responsible for development of neural tissue, or it may simply keep neural cells in a proliferative state while the cells form various neural tissues.

TUMOR SUPPRESSOR GENES

The growth of normal cells is thought to be regulated by growth-promoting cellular oncogenes, whose effects are counterbalanced by growth-constraining genes called tumor suppressor genes. Just as certain cancers appear to be caused by activation of an oncogene, other cancers appear to result from inactivation of a tumor suppressor gene. Mutations that affect a tumor suppressor gene may make that gene unable to oppose the growth-promoting effect of a normal cellular oncogene or of an oncogene introduced by a virus. The existence of tumor suppressor genes has been suspected for a long time, but it has been very difficult to study tumor suppression experimentally, because the action of a tumor suppressor gene only becomes apparent when the gene is missing.

Evidence for the existence of tumor suppressor genes originally came from experiments in which tumor cells were fused with normal cells. When this is done, almost invariably the cells that result are unable to induce tumors in animals and do not behave like tumor cells when grown in culture. This suggests that some feature of the normal cells is able to induce normal growth in the tumor cells. The simplest explanation is that normal cells are contributing genetic information to the tumor cells that leads to suppression of tumor cell growth. The logical conclusion to this line of reasoning is that tumor cells lack this genetic information, perhaps losing it as they transform to the malignant growth habit. The fused hybrid cells, which have double the normal number of chromosomes, are often unstable and frequently shed chromosomes originating from one or the other parent cell line. Occasionally, when a chromosome originating from the normal cell is lost, the hybrid cell can revert to growing like a tumor cell. This phenomenon allowed scientists to identify specific chromosomes associated with suppression of tumor cell growth.

Research involving transfection of whole chromosomes into cultured tumor cells has confirmed that certain chromosomes suppress tumor cell growth. Many different tumor suppressor genes are evidently present in human

and rat cells, since at least 11 different chromosomes can suppress tumor cell growth.²⁰ More than one tumor suppressor gene can suppress the growth of a given tumor, indicating that loss of multiple tumor suppressor genes may be involved in development of certain tumors. One copy of a tumor suppressor gene may be sufficient to suppress the growth of certain tumors, but two copies of a suppressor gene may be required to suppress the growth of other tumors.

Further evidence for the existence of tumor suppressor genes comes from a careful study of the genetics of human retinoblastoma (RB). RB is a rare tumor of the retina that typically affects children under 3 years old. It was observed more than 20 years ago that there were two inheritance patterns for RB. In familial RB, there is a dramatically increased incidence of RB, while in sporadic RB the disease arises without any family history; a “two-hit” model of inheritance was proposed in 1971 to explain these inheritance patterns. However, at the time this model was proposed, there was no knowledge of RB gene function, and it took more than 10 years for the data to catch up with theory. It was not until 1983 that an elegant series of genetic analyses showed that the two genetic targets required by a two-hit model were, in fact, a pair of chromosomes. Thus, the two hits required by the model are the loss of both functional copies of the RB gene on the two copies of chromosome 13 (Fig. 14).

Modern analyses have shown that the RB gene product is expressed at high levels in most tissues of normal subjects. The RB protein is localized in the cell nucleus, where it apparently binds to DNA, suggesting that the RB gene product regulates gene transcription in normal cells. But when RB gene expression was studied in six RB patients, it was found that RB gene expression was totally absent in two of the patients, and was aberrant in the remaining four patients.²³ The gene that was inactivated by loss or mutation was localized to a small region of chromosome 13. Although RB is generally curable if detected early, survivors have a higher than normal risk of developing other cancers later in life, particularly bone cancers. Interestingly, loss of genetic information on chromosome 13 is also associated with osteosarcoma, and this correlation was identified well before the RB gene was localized to chromosome 13.

Mutational inactivation of the RB gene is associated with development of several other cancers besides RB. A study of human breast cancer found that 4 of 10 patients had lost some genetic information on chromosome 13.²⁴ However, this is not proof that the RB gene was inactivated, since part of chromosome 13 could be lost without necessarily interfering with RB gene function. Therefore, human breast cancer cell lines were screened for loss or inactivation of the RB gene, using sophisticated molecular biological techniques. It was found that

22% (2 of 9) of the breast cancer cell lines screened had an inactivated RB gene.

Expression of the RB gene has also been probed in human small cell lung carcinomas (SCLC) cell lines using an antibody directed against the RB protein. Tumor lines established from 20 patients were studied: 7 (35%) of the tumors did not express RB, whereas RB expression was detectable in 13 patients.²⁵ Another study examined RB gene expression in a wide range of human lung cancers and lung cancer cell lines.²⁶ There were mutations affecting the RB gene in 13% of primary SCLC tumors (and in 18% of SCLC cell lines), but no RB mutations were present in non-SCLC lung cancers. Even more convincingly, it was found that the RB gene product was entirely absent in 60% of the SCLC cell lines, while this gene product was present in 90% of all non-SCLC cell lines. These findings strongly support the conclusion that RB gene inactivation is involved in development of SCLC.

The tumor-suppressing action of the RB gene was clearly demonstrated in a recent series of experiments.²⁷ Human RB cells were injected into the eye of an immune-incompetent mouse, where they formed tumors with histological features similar to that of RB. But if those RB cells were first transfected with a normal, functional RB gene, then tumor formation was blocked. Thus, tumors only formed when the RB gene was inactivated, and presence of a normal RB gene was sufficient to suppress tumor formation. This suggests that transfection of retinal cells with a normal RB gene may eventually be a viable therapeutic strategy for children afflicted with RB.

Although the concept of tumor suppression was first elucidated for the RB gene, another tumor suppressor gene, called p53, is already documented as the most frequently mutated gene in human cancer. The p53 gene is thought to encode a protein that plays a critical role in controlling the cell cycle, since it is known that this protein binds DNA and can activate gene transcription.²⁸ The p53 protein is usually found in the cell nucleus, and simple mutations of this protein make it carcinogenic. At least 30 different mutations of p53 have been identified, with the various mutations producing proteins that range from mildly dysfunctional to strongly carcinogenic. Many of the mutations affecting the p53 gene result in a p53 gene product that is very stable, whereas the normal p53 gene product is unstable. Thus, the p53 gene product accumulates and can be detected in some tumor cells, while it does not accumulate in normal cells.²⁹

The Li-Fraumeni syndrome, a familial cancer syndrome associated with increased susceptibility to a wide range of cancers, is apparently caused by a mutation of the p53 gene.³⁰ This syndrome is of great interest because of the

diversity of childhood and adult tumors that occur in affected individuals. A model of the inheritance of this cancer predicts that the probability of developing any cancer reaches nearly 50% by age 30, when only 1% of the general population have developed cancer. But the rarity and high mortality of Li–Fraumeni syndrome has rendered traditional methods inadequate to identify the gene responsible for this syndrome. An alternative approach to this problem was simply to select a plausible candidate gene and screen affected family members for mutations of that gene. The tumor suppressor gene p53 was studied in this way, because of evidence that this gene is inactivated in several of the cancers that are associated with Li–Fraumeni syndrome. Mutations of p53 were detected in all five Li–Fraumeni syndrome families that were analyzed. Of the nine individual family members with a p53 mutation, seven died of a cancer typically associated with Li–Fraumeni syndrome.³⁰ However, the frequency of p53 gene mutations in the general population is unknown, so the association between p53 gene mutation and the Li–Fraumeni syndrome cancer is somewhat weakened. Nevertheless, it may eventually become possible to screen for mutations of p53, to identify individuals with a high cancer risk.

Mutations of the p53 gene have been identified in a broad range of cancers, and these mutations are not limited to people from Li–Fraumeni syndrome families. For example, genetic mutations of p53 were identified in 62% (8 of 13) of the uterine carcinoma cell lines examined.³¹ Similarly, when 31 ovarian cancers were examined for changes affecting p53, loss of the gene was detected in 16 cases (52%) and mutation of the gene was detected in 9 cases (29%). Only 32% of the tumors showed neither mutation nor loss of p53, suggesting that alteration of p53 plays an important role in ovarian cancer development.³² A study of tissue samples from colorectal tumors found that 70% of these tumors showed abnormal expression of the p53 gene.²⁹ Of those colorectal tumors that showed elevated expression of the p53 gene, about 76% also showed a loss of genetic information affecting chromosome 17. Since this is the chromosomal location of the p53 gene, it indicates that many of the tumor cells were only able to express a mutated version of the p53 gene.

A large number of human melanomas, at varying stages of tumor progression, have been tested for expression of mutant p53 protein.³³ Eighty-five percent (45 of 53) of the tumors tested, which came from a range of primary and metastatic melanomas, had detectable mutations of p53. There was a significant increase in the prevalence of p53 mutations as the tumors grew, and there was a greater proportion of p53 mutations in metastatic tumors, compared with primary melanoma. Dysplastic nevi are small pigmented lesions that are

often precursors to malignant melanoma, yet no (0 of 3) dysplastic nevi showed mutations of p53. In the small primary melanomas examined, 60% (3 of 5) showed p53 mutations, while in large primary melanomas, 73% (11 of 15) showed p53 mutations. In a sample of metastatic melanomas, 94% (31 of 33) showed p53 mutations.³³ This finding clearly shows that loss of p53 is associated with progression of malignant melanoma.

Mutations of the p53 gene are also frequently found in cell lines derived from patients with active T-cell acute lymphoblastic leukemia (T-ALL) and in cells of some patients in relapse.³⁴ A T-ALL cell line that lacks p53 protein was transfected with a normal p53 gene, to examine the feasibility of suppressing tumor growth in T-ALL patients. Expression of the p53 protein reduced the growth rate of infected cells in culture, and prevented these cells from forming tumors in immunodeficient mice. This suggests that suppression of acute leukemia cell growth may become a viable therapeutic strategy for T-ALL, if the normal p53 gene can be introduced into tumor cells with high efficiency.

The two best-known tumor suppressor genes, p53 and RB, show several similarities. Both are growth-suppressing proteins, both are found in the cell nucleus, and evidence is accumulating that both proteins must be eliminated for cell transformation to occur. Therefore, p53 and RB may serve complementary functions in growth regulation. A potential interaction between mutations of p53 and RB was identified in a type of liver cancer called hepatocellular carcinoma. The p53 gene was examined in 43 primary hepatocellular carcinomas, 22 of which were advanced cancers, and 21 of which were early stage cancers.³⁵ Abnormalities of the p53 gene were detected in 36% (8 of 22) of the advanced tumors, but in none of the early cancers. Loss of the RB gene was seen in 6 of the 8 tumors with a p53 mutation, suggesting that loss of both tumor suppressor genes may be involved in progression of hepatocellular carcinoma.

Although we have discussed only two tumor suppressor genes in depth, evidence has accumulated that there are several other important tumor suppressor genes. These include: the DCC (deleted in colorectal carcinoma) gene, loss of which is associated with progression of colon cancer; the neurofibromatosis tumor suppressor (NF-1) gene, loss of which is associated with benign nerve tumors and with malignant neurofibrosarcoma; the Wilms' tumor (WT-1) gene, loss of which is associated with a kidney tumor; the adenomatous polyposis coli (APC) gene, loss of which may be associated with familial colon cancer; and perhaps also the *erbA* gene, loss of which may be associated with certain leukemias.

Tumor suppressor genes could suppress tumor formation through a range

of different possible gene actions. For example, tumor suppressor genes could: induce terminal cell differentiation; maintain stability of the cellular genome; trigger cell death in transformed cells; act as a transducer for negative growth factors; modulate the display of antigens on the cell surface; regulate angiogenesis by the tumor; facilitate cell-to-cell communication; or alter gene expression.

Clearly, a better knowledge of tumor suppressor gene function is potentially of major clinical importance. If sensitive tests can be developed to determine which patients and which tumors show loss of tumor suppressor genes, then it will be possible to determine which patients are at greater risk of tumor recurrence and which patients should receive the most aggressive therapy. Ultimately, a thorough understanding of the function of tumor suppressor genes may permit physicians to suppress the growth of a tumor even after it has become established. We have seen exciting hints of this in the experiments showing arrest of RB growth after transfection of cells with a normal RB gene, and in the suppression of T-ALL following introduction of a normal p53 gene into tumor cells.

CHAPTER 5

Tumor Energy Metabolism

All living cells undergo processes of chemical transformation, the sum total of which is called metabolism. Cells obtain all of the energy they need to live and grow from the complex, carefully controlled chemical transformations of metabolism. Quite literally, life is chemistry. In fact, the chemistry of metabolism in all kinds of cells is so similar that this provides one of the strongest possible arguments for Darwinian evolution. The manifest similarities between the cellular metabolism of mammals and reptiles, for example, show that at one time these cells must have shared a common ancestor. Thus, it should not be surprising that human tumor cells have an energy metabolism that is similar to that of normal human cells, with a few important differences.

WHY STUDY TUMOR METABOLISM?

A clear understanding of tumor metabolism is of potentially great importance, because such an understanding may reveal vulnerabilities in the tumor cell that can be exploited in the successful treatment of cancer. There are several additional reasons why a thorough understanding of the metabolism of an individual tumor could be important in tailoring therapy for that tumor:

1. The sensitivity of a tumor to many chemotherapeutic agents is determined by the rate of cell growth and the proportion of cells in the tumor that are actually growing. Rapidly growing tumors are more sensitive to radiation or chemotherapy, yet it is often difficult to determine which tumors are growing rapidly.
2. The amount of oxygen present within a tumor determines the success of radiation therapy, since radiation kills tumor cells by creating activated (“singlet”) oxygen molecules, which are cytotoxic. Since the

- amount of oxygen within a tumor also affects cell metabolism, characterizing cell metabolism may give insight into tumor oxygenation.
3. Knowledge that metabolism of a particular substrate is increased could allow a physician to target a radioactively labeled molecule to the tumor. This molecule could be used to kill the tumor cell, or it could be used to discriminate tumor from normal tissue in a medical image.
 4. The pH of a tumor, which is determined by cellular metabolism, may determine how well a particular chemotherapeutic agent is taken up by tumor cells, since drug transport into a cell can be pH dependent, or it may determine the effect of the drug on the cell.

Until quite recently, it was not possible to study the metabolism of human tumors without either potentially harming the patient or surgically removing the tumor. However, recent development of certain very sophisticated research tools now allows the physician to characterize tumor metabolism directly, without causing harm to the patient. The techniques of positron emission tomography (PET), magnetic resonance imaging (MRI), and magnetic resonance spectroscopy (MRS) have each provided compelling new insights into the metabolism of human tumors. It is beyond the scope of this chapter to provide an in-depth explanation of these techniques, since each technique must be understood in terms of some very complex physics. Suffice it to say that each technique can provide unique insights into the metabolism of tissue, and each can do so without requiring any particularly painful or arduous procedures for the patient.

PET is capable of measuring the rate of uptake of a particular radioactively labeled chemical by a tissue. By varying the chemical that is labeled, and by using sophisticated mathematical techniques, it is possible to use PET to measure tissue oxygen or glucose utilization, blood flow, blood volume, drug uptake, or tissue pH. MRI is capable of generating medical images that are sensitive to a variety of parameters that affect the state of water within a tissue. By changing the way MRI data are collected, information can be obtained on tissue structure, arterial blood flow, capillary perfusion, tissue water content (edema), oxygenation of arterial blood, or the distribution of ions within a tissue. MRS is capable of analyzing the chemical state of various atoms within living tissue, so it is possible to measure the concentration of the major metabolites within living tissue. This can enable researchers to characterize tissue biochemical composition, energetic state, and pH, or to measure the rate of drug delivery to a tissue.

For technical reasons, much of the most recent research on human tumor metabolism has been done on brain tumors, so the following discussion will be weighted toward a consideration of these tumors. However, it should be noted that brain tumor metabolism may well be representative of the metabolism of other solid tumors.

METABOLISM OF NORMAL CELLS

In order to understand tumor cell metabolism, it is first necessary to understand the metabolism of normal cells. Human and animal cells are capable of metabolizing glucose, by breaking it down to its constituent parts. Metabolism of any substrate requires the cooperative action of many different proteins called enzymes, whose action catalyzes, or stimulates, chemical reactions. While glucose is certainly not the only molecule that can be broken down by a cell, it is the one most commonly metabolized in normal cells. Ultimately, in the complete metabolic breakdown of one molecule of glucose, the only end products are carbon dioxide, water, and energy. During metabolism, a great deal of energy is liberated from the glucose molecule, and this energy can be stored in the form of a molecule known as ATP. In essence, ATP is the energy currency of the cell, since it is used to drive virtually all energy-requiring processes, and it is produced in virtually all energy-liberating processes.

Metabolism is generally divided into two parts, anabolism and catabolism. Anabolism is the set of chemical reactions in which simple molecules are built up into complex molecules, often at the cost of a great deal of energy. Catabolism is the set of chemical reactions in which complex molecules are broken down into simple molecules, often with the release of a great deal of energy. To a certain degree, the dichotomy between anabolism and catabolism is artificial. Both processes can occur simultaneously in a single cell, and both processes are required if a cell is to survive. Small molecules, the products of catabolism of one complex molecule, can immediately enter an anabolic series of chemical reactions and be used to synthesize another complex molecule. Whether an anabolic or a catabolic metabolic pathway is activated depends on the changing needs of the cell, and the activity of different pathways can be modulated from moment to moment.

Cells obtain their requirement for energy from the catabolism of glucose and certain other molecules. Insight into the rate of glucose catabolism can be

obtained by using PET to measure the rate at which a tissue takes up radioactively labeled glucose from the bloodstream. The tissue is unable to distinguish between the radioactively labeled glucose and a normal glucose molecule, so the rate at which the labeled molecule is taken up is related to the rate at which nonlabeled glucose is taken up. If a tissue takes up a great deal of labeled glucose from the bloodstream, it is assumed to have a high metabolic rate, whereas if a tissue takes up small amounts of glucose, it is assumed to have a low metabolic rate. This may be a simplistic assumption, since a cell can take up more glucose than it actually needs, yet PET has been shown to give accurate estimates of tissue metabolic rate. The advantage of PET is that it can give an estimate of metabolic rate in as little as an hour without doing surgery on the patient.

When a molecule of glucose is taken up by a cell, it is usually catabolized to obtain the energy stored within it. A molecule of glucose is broken down as it moves sequentially down two metabolic pathways: first glycolysis, then the Krebs cycle (which in this discussion includes the electron transport chain) (Fig. 17). The first metabolic pathway, glycolysis, uses enzymes present in the cell cytoplasm. Glycolysis can occur in the total absence of oxygen, since it only requires glucose as a substrate. The second metabolic pathway, the Krebs cycle, uses enzymes found within tiny structures in the cell cytoplasm called mitochondria. The Krebs cycle requires oxygen in addition to the substrates supplied by glycolysis. This oxygen is obtained from the air we breathe and is carried to individual cells in the bloodstream. As a point of fact, the Krebs cycle is the only reason we need to take in oxygen; other than the electron transport chain of the Krebs cycle, there are no significant oxygen-requiring processes in the body. Since the Krebs cycle occurs in mitochondria, these tiny organelles are often referred to as the powerhouse of the cell.

Glycolysis is a process whereby a single molecule of glucose is broken down into two smaller molecules, with the release of a certain amount of energy. The energy released from glucose can be stored as ATP. The end products created during glycolysis ordinarily feed into the Krebs cycle, where a great deal more energy can be released during their continued catabolism. But if the Krebs cycle is prevented from occurring, the end product of glycolysis is lactic acid. A cell that lacks oxygen is unable to catabolize glucose by the Krebs cycle, so this cell may be forced to release lactic acid as an end product of glycolysis.

The total energy yield from catabolism of a molecule of glucose is determined by the way the glucose is catabolized. Glycolysis can release (and

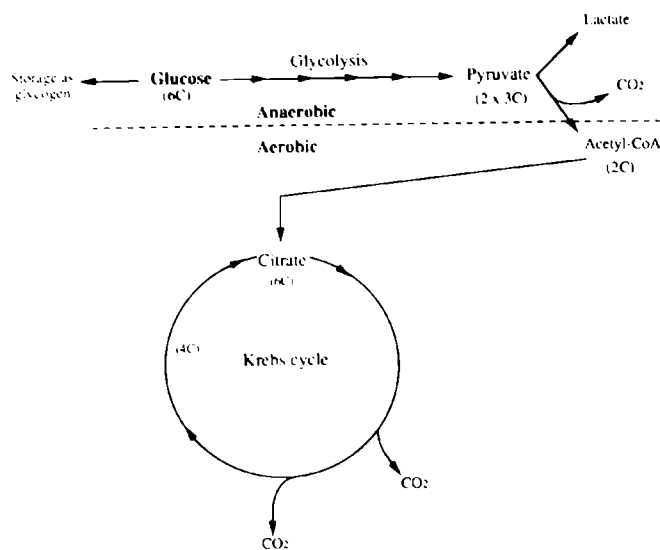


Figure 17. Summary diagram of cellular metabolism, showing how glycolysis and the Krebs cycle relate to one another. Glucose, a six-carbon (6C) molecule, is broken down by sequential reactions to two 3C molecules (pyruvate), which can then feed into the Krebs cycle. In the Krebs cycle a 2C molecule (acetyl-CoA) is combined with a 4C acceptor molecule to make a 6C molecule (citrate), which then loses two carbons (as CO₂) to regenerate the acceptor molecule. Oxidative phosphorylation is not shown, but would involve the stepwise oxidation of reduced molecules which are generated primarily during the Krebs cycle. The dashed line indicates that glycolysis (above the line) can proceed in the absence of oxygen (i.e., anaerobic conditions), but that the Krebs cycle requires oxygen (i.e., aerobic conditions).

store as ATP) only about 2% of the energy in a molecule of glucose, so the efficiency of this process is quite poor. If a molecule of glucose undergoes glycolysis without also undergoing the Krebs cycle, then 98% of the energy within the glucose molecule is wasted by the cell. However, the Krebs cycle is capable of storing as ATP about 36% of the energy in a molecule of glucose. Clearly, the Krebs cycle is far more efficient at extracting energy from a molecule of glucose. If a cell is to obtain the maximal energy yield from catabolism of glucose, then both glycolysis and the Krebs cycle must occur, in a very closely coupled fashion. A cell that succeeds in performing both glycolysis and the Krebs cycle will be able to store 38% of the total energy in a molecule of glucose as ATP. But if the coupling between glycolysis and the Krebs cycle breaks down, then some products of glycolysis may not feed into the Krebs cycle and a certain amount of energy will be lost by the cell.

BLOOD FLOW IN NORMAL BRAIN AND BRAIN TUMOR

So far we have discussed cellular metabolism as if each cell had access to a limitless supply of the necessary substrates. Yet in tissues, as everywhere else, resources are often limiting, and cells can lack for sufficient amounts of oxygen, glucose, or other substrates. Many disease states in humans are associated with an acute or chronic deficiency of some substrate, often oxygen. For example, stroke is a disease state in which a part of the brain suffers an interruption of blood flow, so that both oxygen and glucose become limiting; for various reasons, it is usually thought that oxygen is more limiting than glucose. Because of interrupted blood flow, part of the brain may actually die, leaving the patient in a state of neurologic deficit. Similarly, myocardial infarction is a sudden insufficiency of blood supply to a part of the heart, which can lead to death of that part of the heart muscle. Infarction, the interruption of blood supply to a tissue, can affect various other tissues, including the spleen, kidney, intestine, lung, or testes.

The metabolic rate of a tissue is generally closely coupled to the rate of blood flow in that tissue. Thus, the brain, which has a high metabolic rate compared with most other tissues, has a high and constant rate of blood flow. Blood flow in the brain, measured by PET, averages 45 ml of blood per 100 ml of tissue per minute in the gray matter, a type of brain tissue with a very high metabolic rate.³⁶ The total volume of blood in gray matter is about 4.1 ml of blood per 100 ml of tissue. Therefore, blood flow through the gray matter is so rapid that the blood vessels can effectively fill and empty 11 times in a single minute (i.e., $45/4.1 = 11.0$). In white matter, a type of brain tissue that has a relatively low metabolic rate, blood flow averages 27 ml of blood per 100 ml of tissue per minute. But the blood volume of white matter is only 2.4 ml of blood per 100 ml of tissue, so blood flow through the white matter is rapid enough that the blood is actually replenished more than 11 times a minute.

The perfusion rate of brain tumors tends to vary widely, but it is generally somewhat lower than that of normal brain tissue. For example, the rate of perfusion measured in a series of 14 brain tumors was 28 ml of blood per 100 ml of tissue per minute.³⁶ The total volume of blood in these tumors was high, so the relative turnover rate of tumor blood was somewhat lower than normal brain. Blood volume measured for these tumors was 4.1 ml of blood per 100 ml of tumor, so blood flow through the tumors replenished tumor blood about 7 times a minute. While this rate may seem quite high, in fact there are cells within the tumor mass that get an insufficient supply of glucose or oxygen. In

general, blood flow in human tumors is highly variable but tends to be less than blood flow in the comparable normal tissue.³⁷

Inadequate tumor perfusion is probably the result of several interacting factors. The total volume of blood in a tumor is usually lower per unit volume than in a corresponding normal tissue. Furthermore, tumor blood vessels are often malformed and aberrant, and may even form shunts that bypass much of the tumor volume. Vessel malformation may arise because new blood vessels supplying the tumor are induced to grow very rapidly, or because these vessels may be unable to grow into an established tumor mass. The tumor can grow so rapidly that it literally outstrips the growth rate of its own vasculature. For these various reasons, some parts of the tumor may have an adequate blood supply while other parts of the tumor may have a poor blood supply. This results in a great deal of heterogeneity within the tumor, with some tumor cells growing rapidly, while cells in other parts of the same tumor may be dying from lack of oxygen or glucose.

But inadequate or inhomogeneous tumor blood flow is not simply the result of malformed or aberrant blood vessels within the tumor. There is accumulating evidence that tumor blood vessels are far more leaky than normal blood vessels, so that fluid leaks out of the vessels and causes the tumor tissue to swell. One recent study showed that the blood vessels in a rat tumor were 10 to 1000 times more leaky than normal rat blood vessels.³⁸ This high degree of vessel leakiness means that a great deal of fluid can potentially leak from the bloodstream into the tumor. Vessel leakiness probably accounts for the large regions of edema, or tissue fluid, that are usually seen during MRI of human brain tumors (Fig. 18). The steroid dexamethasone, which is frequently used in therapy for edema and intracranial pressure in brain tumor patients, has been shown to reduce blood vessel permeability within tumors.

Areas of edema are often found adjacent to brain tumors, so edema is presumably caused by leakage of fluid from tumor blood vessels. As edema fluid accumulates within the tumor, it will percolate out, creating a bulk flow of fluid away from the tumor core. In the closed volume of the cranium, if the space taken by edema fluid increases, then the blood space must decrease, and flow of blood through the vasculature will be hindered. A PET study of blood flow through areas of edema in normal brain has clearly shown this reduction in blood flow. Blood flow in edematous normal brain averaged 16 ml of blood per 100 ml of tissue per minute, which is less than half the normal blood flow rate of gray matter.³⁶ The total volume of blood in edematous brain was 2.2 ml of blood per 100 ml of tissue, so blood flow through edematous normal brain



Figure 18. A large cerebral mass in a female patient (43 years old), which was later diagnosed as glioblastoma multiforme. This is a magnetic resonance image (MRI), which shows the tumor as a large, dark, oval mass at the posterior of the cerebral hemisphere (lower left). The tumor is surrounded by a bright area, which is brain tissue made edematous by leakage of fluid from the tumor. Note that brain structures are displaced to the right in the image because of the large mass of tumor and edematous brain. (Photograph courtesy of Dr. Kenneth Maravilla and Dr. James A. Nelson, University of Washington School of Medicine.)

replenished blood only 7 times a minute. Thus, the fluid pressure developed around a tumor (resulting from edema) can cause a decrease in blood flow to otherwise-normal brain.

Recently the fluid pressure within a range of human tumors was measured. Fluid pressure in tumors of patients with cervical cancer averaged about tenfold higher than that in normal cervix.³⁹ In patients with cervical cancer, the availability of oxygen within the tumor was inversely correlated with fluid pressure, so that patients with elevated fluid pressure had low levels of tumor oxygenation. Similarly, the fluid pressure in metastatic melanoma of the skin was about tenfold higher than normal fluid pressure in skin.⁴⁰ An elevation of fluid pressure as profound as this within a tumor is expected to virtually shut down blood flow through the tumor.

CONSEQUENCES OF INADEQUATE BLOOD FLOW IN TUMORS

As in all cells, the energy required by tumor cells is obtained by breaking down substrates taken up from blood. Tumor cells with a chronic insufficiency of blood supply may also have a chronic insufficiency of oxygen or glucose. While it is very difficult to measure levels of glucose within human tumors, the amount of oxygen present in a range of human malignancies has been measured, using tiny oxygen electrodes that are inserted directly into the tumor. A large body of evidence shows that human tumor oxygenation is often markedly lower than the oxygenation of corresponding normal tissue. Understandably, this sort of evidence is not readily available for human brain tumors, because it is not possible to insert needle electrodes repeatedly into normal brain as a comparison with brain tumor. But in tissues where this comparison can be made, tumor oxygenation is almost always much lower than oxygenation of normal tissue.

For example, the average oxygen level in tumors of the cervix is dramatically lower than that of normal cervix.³⁷ Average oxygen tension of normal cervix is 36 mm Hg (the units are a way of expressing oxygen pressure, analogous to a barometric pressure reading). Early (Stage 0) cervical cancer has a mean oxygen tension of 20 mm Hg. Stage I cervical cancer has a mean of 13 mm Hg, and Stage II, 5 mm Hg. Thus, oxygen tension of normal cervix is more than 7 times higher than in a high-grade cervical cancer, and tumor oxygenation decreases sharply with increasing tumor malignancy. Similarly, oxygen tension of normal breast is 1.4–4.4 times higher than in breast tumors, oxygen tension of skin is more than 6 times higher than in melanoma, and oxygen tension of muscle is 2.8–6.3 times higher than in soft tissue sarcomas.³⁷

The major metabolic consequence of inadequate blood flow to tumors is a poor energy state of those tumors. MRS has been used to observe the energy state of human tumors, since MRS is capable of characterizing the amount of cellular ATP. A good deal of accumulated evidence shows that the energy state of most human tumors is poor relative to normal tissue of the same type.⁴¹ For example, the brain typically has high levels of ATP and of a second compound called phosphocreatine (PCr), which enables brain cells to store energy. But when MRS is used to examine the energetic state of human brain tumors, there is a distinct deficit of both ATP and PCr compared with normal brain. In fact, PCr may be entirely absent in some tumors, while ATP may be reduced by half with respect to normal brain. But a critical question remains as to whether

oxygen or glucose deprivation is more limiting for these tumor cells. This is a difficult question, since inadequate tumor blood flow could mean that both oxygen and glucose are limiting (Fig. 19).

Determining whether oxygen or glucose is more limiting to tumor cells might provide insight into a therapeutic approach to cancer. If chronic insufficiency of glucose prevents some tumor cells from metabolizing substrates by glycolysis or the Krebs cycle, then obtaining energy for cell metabolism would be virtually impossible. These cells could perform only the most basic metabolic functions, and might enter a state of depressed metabolism that could make them resistant to therapy. Yet if chronic insufficiency of oxygen prevents tumor cells from using the Krebs cycle, but does not prevent them from performing glycolysis, the result could be quite different. These cells would be left with the inefficient process of glycolysis as the only ready source of energy, but they might still have a supply of energy sufficient for their purposes. The energy state of these tumors might seem poor by MRS if the ATP made in glycolysis was used immediately in coupled reactions. But these cells might be metabolically more active than if glucose were limiting, so they might be vulnerable to a therapy directed against an active cell metabolism.

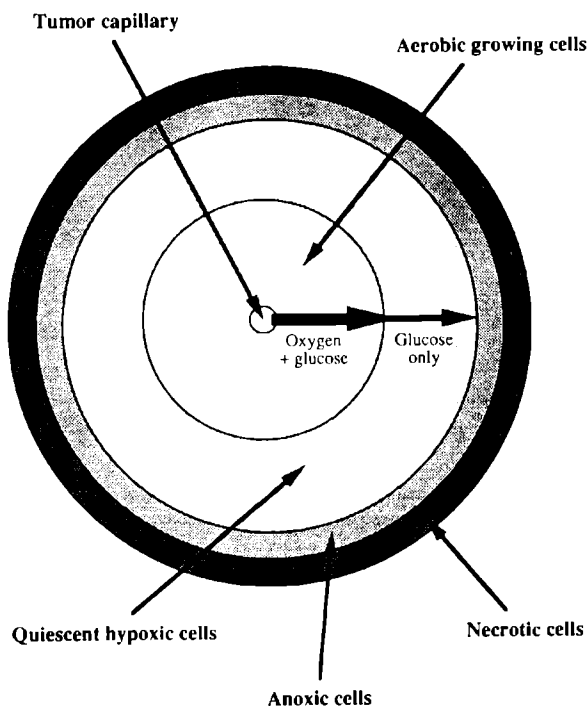


Figure 19. Schematic diagram of the probable pattern of growth of tumor cells around a tumor capillary. Only the aerobic cells immediately surrounding the capillary are actively growing because they have an adequate supply of both oxygen and glucose. At a greater distance from the capillary are viable hypoxic cells, quiescent because of lack of adequate oxygen. At a still greater distance are anoxic cells, which lack adequate supplies of either oxygen or glucose, but which have not yet died. At the greatest distance from the capillary are necrotic cells, which have died as a result of chronically inadequate nutrient supply.

To imply that tumor cells resort to glycolysis only because they have an insufficient supply of oxygen is false; the situation is more complex than that, and is actually not at all well understood. About 30 years ago, an experimental system was developed to study tumor oxygen consumption. A small number of tumor cells were injected into the ovary of a rat, and these cells grew to replace the normal cells of the ovary without disturbing blood flow to the organ. The end result of this process was that the rat ovary, which normally is tiny, was completely replaced with tumor tissue weighing several hundred times more than the ovary. Nevertheless, this tumor tissue was still supplied by one artery and drained by one vein. Scientists could learn much about tumor metabolism by identifying and measuring the various chemicals in the blood flowing into and draining from the tumor. Any difference between the arterial and venous blood could only be caused by tumor metabolism. If a chemical is present in arterial blood but absent or reduced in venous blood, then that chemical must have been taken up by the tumor. Conversely, if certain chemicals are absent from arterial blood but present in blood draining the tumor, these chemicals must have been released by the tumor.

Researchers were able to show that tumor cells use oxygen. Yet, despite the availability of oxygen, tumor cells produced lactic acid, which is normally an end product of cells lacking oxygen. The fact that tumor cells produce lactic acid even when oxygen is available shows that glycolysis is not used solely to compensate for the absence of oxygen. Evidently, tumor cells preferentially perform glycolysis. Yet tumor cells that release lactic acid do not necessarily have a growth advantage over tumor cells that do not have a high level of glycolysis.

PET has been used to measure uptake of radioactive oxygen by human brain tissue and brain tumor. If normal tissue is temporarily subjected to a deficit of oxygen by decreasing the rate of blood flow, then the efficiency with which oxygen is taken up from the bloodstream increases sharply. For example, in normal brain tissue, about 40% of the oxygen present in the blood is taken up by brain as blood moves through the brain tissue.³⁶ If blood flow to the brain is too slow to meet normal metabolic demands, then the oxygen-starved brain responds by extracting virtually 100% of the oxygen in blood. But if oxygen extraction by brain tumors is measured by PET, a very different picture emerges. Cerebral tumors may extract as little as 19% of the oxygen in blood, even though these tumors are poorly perfused. This is a much lower oxygen extraction efficiency than is found even in well-perfused normal brain. It is a consistent finding in brain tumors that the rate of extraction of arterial oxygen is

low, as is the rate of oxygen utilization, even though tumor perfusion is poor. This appears to be a profound paradox: the tumor is oxygen-starved because it is poorly perfused, yet it responds to this starvation by being less efficient at extracting oxygen from the blood. This is as if a person dying of thirst in the desert forgot how to drink.

If PET is used to measure glucose utilization by normal brain, and by brain tumor, the results are strikingly different (Fig. 20). The fraction of glucose extracted from arterial blood by brain tumor is similar to the fraction of glucose extracted by normal brain.⁴² This similarity in glucose utilization occurs despite the fact that brain tumor extraction of oxygen is low compared with normal brain. The rate of glucose utilization by brain tumors is related to the degree of malignancy of the tumor, since fast-growing, aggressive tumors have an elevated rate of glucose utilization. Similarly, PET measurements in human breast carcinoma show that utilization of glucose by breast tumors is

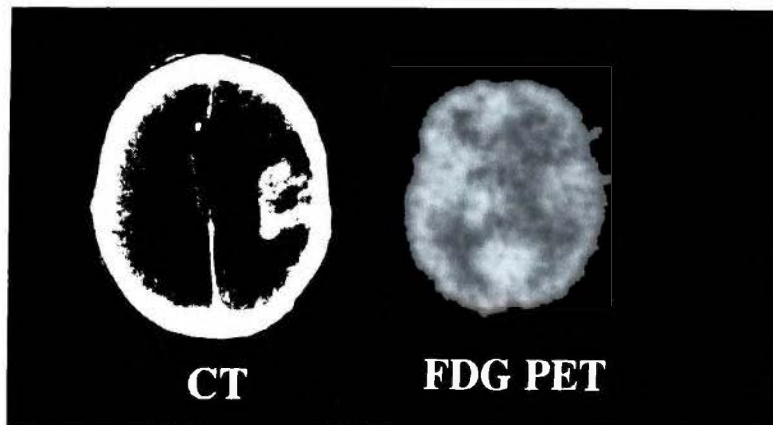


Figure 20. X-ray computed tomographic (CT) image and positron emission tomographic (PET) image of a 49-year-old male patient with recurrent brain glioma. The CT image shows that tumor occupies a large volume on the right side of the brain, with structures at the midline pushed into the left cerebral hemisphere by the tumor volume. The PET image was obtained by infusing a radioactive analogue of glucose (^{18}F -fluoro-deoxyglucose) into the blood and imaging the location of radioactivity several minutes later. This analogue of glucose accumulates in cells, because it is taken up across the cell membrane in the same way as glucose, but cells are unable to further metabolize it. The PET image, which is brighter in the tumor, shows that tumor takes up more glucose than surrounding tissue on that side of the brain, probably because glucose metabolism by tumor is inefficient. However, note that glucose uptake by the entire right side of the brain is lower than by the left side of the brain, probably because blood flow to the right side of the brain is reduced by intracranial pressure. (Photograph courtesy of Dr. Michael Graham, University of Washington School of Medicine.)

greater than utilization of oxygen by these tumors.⁴³ The reason why tumor cells utilize glucose, yet are unable to utilize oxygen, is poorly understood. If the absence of oxygen simply blocked the Krebs cycle, then this should eventually depress the rate of glycolysis, since the two processes are tightly coupled in normal tissue. Apparently, glycolysis is somehow uncoupled from the Krebs cycle in tumor tissue, so the rate of glycolysis is no longer regulated by products from the Krebs cycle. But the means by which this uncoupling takes place remains unclear.

CHANGES IN CELLULAR METABOLISM IN TUMORS

One of the most consistent findings by scientists studying the biochemistry of tumor cells is that, compared with normal cells, tumor cells show an increase in the catabolism of glucose and an increase in the production of lactic acid. But this altered metabolism is not exclusively a hallmark of cancer, since a number of normal tissues can also show an elevated rate of glycolysis, even in the presence of oxygen. For example, cells of the intestinal lining, the retina, and the renal medulla of the kidney all perform aerobic glycolysis on a regular basis, and many cells that are stimulated to proliferate by hormones can show a temporary elevation of glycolytic rate.

The benefit of glycolysis to the tumor is that tumor cells can obtain an abundant source of energy even though they may be unable to carry out the Krebs cycle. But this benefit to the tumor is won at the expense of the patient. As noted, glycolysis without the Krebs cycle is an inefficient way of catabolizing glucose. So, while the tumor may gain relatively little from catabolizing a molecule of glucose, the patient potentially loses a great deal of energy that could have been obtained through complete catabolism of the glucose molecule. Usually, this energy is not totally lost to the patient, because normal liver can recover lactic acid from the bloodstream and use it to resynthesize glucose. But resynthesis of glucose is a relatively expensive process in energetic terms, so the incomplete catabolism of glucose done by most tumors is a very wasteful process.

Increased glucose catabolism is not consistent in all tumor cells, being seen primarily in tumor cells with a rapid growth rate and an anaplastic growth form. An increased rate of glucose metabolism is usually associated with rapid growth of tumor cells, since glycolysis can provide cells with an abundant source of energy. In fact, up to 65% of the energy production of some highly

malignant tumor cells is provided by the glycolytic pathway. Yet there is another advantage to tumor cells from having such an abnormally high rate of glycolysis: glycolysis produces an abundant supply of a precursor molecule needed to synthesize DNA.

A clue as to how tumor cells utilize glucose without also using oxygen has come from studies of tumor cell structure. It has been observed that tumor cell mitochondria frequently differ, both structurally and functionally, from mitochondria in normal cells. In addition, in anaplastic tumor cells, the number of mitochondria per cell can be dramatically reduced. In some liver tumor cells grown in culture, the number of mitochondria per cell is reduced by as much as 80% relative to the number of mitochondria in a normal liver cell.⁴⁴ Generally, the more malignant the tumor cell, the fewer mitochondria there are within that cell. A cell with a reduced number of mitochondria might be unable to use oxygen, even if oxygen were abundant.

Abnormally high rates of glycolysis in tumor cells may also be caused by changes in the abundance of certain enzymes involved in glucose catabolism. There are often marked changes in tumor cells regarding the distribution and abundance of an enzyme called hexokinase. Normally this enzyme, which is involved in glycolysis, is found free in the cytoplasm. But in highly malignant tumor cells, hexokinase activity is increased, and as much as 65% of the enzyme actually attaches to the surface of the mitochondria. Elevated levels of hexokinase seem to be required if tumor cells are to show a high rate of glycolysis. The attachment of hexokinase to the mitochondria apparently prevents hexokinase from being inhibited in its action, and may also help to ensure a ready supply of energy for the enzyme. This could potentially have profound effects on cellular energy production.

TUMOR pH

Tumor pH is an important parameter of tumor biology because it can affect tumor response to therapy. Acidity within a tumor decreases tumor cell radiosensitivity, modulates the toxicity of chemotherapeutic drugs, and enhances cell killing by hyperthermia. There has been considerable interest in characterizing tumor pH, to determine the most effective therapy for a given tumor.

Since tumor cells have an elevated rate of glucose catabolism and lactic acid production, it has long been thought that tumor pH should be acidic. This

is a reasonable idea because if tumor cells do produce large amounts of lactic acid, which is a moderately strong acid, then this should make local tumor pH more acidic than normal. Early research using miniature pH electrodes inserted into human tumors during surgery, seemed to confirm this hypothesis.

The average pH of human glioblastomas measured by microelectrode is about 6.9, meaning that these tumors are somewhat acidic (i.e., pH 7.0 is referred to as neutral pH). By contrast, the average pH of normal brain, measured by the same technique, is about 7.1, or slightly above neutral pH (i.e., normal brain is alkaline). But the pH electrodes used to make these measurements are very large relative to the dimensions of a cell, so a pH electrode inserted into tissue is averaging across a large number of cells. The pH measured by electrode is thus a mean value of pH measured inside many cells and pH measured outside those cells, and the pH inside a cell may be quite different from that outside the cell. Furthermore, insertion of a needle electrode into tissue can disrupt blood flow and can actually destroy some cells.

When tumor pH is measured by a different technique, such as MRS, quite different results are obtained, which raises doubts about the validity of the pH electrode results. For technical reasons, it is thought that MRS is largely sensitive to the average pH within cells, and that pH of the fluid outside the cells makes only a minor contribution to the MRS measurement of pH. The average pH of brain tumors, as measured by MRS, is about 7.1, while the average pH of normal brain is about 7.0. Thus, brain tumors are actually significantly more alkaline than normal brain. When pH values for brain and for brain tumor are measured by PET, the PET values are comparable to the MRS values. Evidently the discrepancy between electrode measurements and MRS measurements is the result of the different tumor volumes sampled: electrodes sample inside and outside the cells, while MRS (and PET) sample mostly inside the cell.

These results suggest that the pH inside tumor cells is somewhat more alkaline than outside the cells. This has been explained in a number of different ways, but the important point is that this finding came as a real surprise to scientists. It is unclear whether this particular insight will lead to a significant therapeutic advance against cancer. However, scientists are driven by the hope that a better understanding of cancer will eventually lead to more successful treatments for the disease.

CHAPTER 6

Tumor Invasion and Metastasis

The hallmark of malignant cancer, and that aspect of cancer that makes it most frightening and difficult to cure, is that malignant tumors can be both locally invasive and widely metastatic. A locally invasive tumor is one that infiltrates into surrounding tissues, sending out fingers of cells that penetrate into the mass of cells surrounding the tumor. Thus, normal tissue can have strands of cancerous cells running through it, each cell of which is potentially capable of forming a new tumor if the original tumor mass is surgically removed. A metastatic tumor is one that is capable of seeding cells into tissues that are at a distance from the original tumor, where these cells initiate the formation of secondary tumors. Occasionally a tumor will be so widely metastatic that, by the time it is diagnosed, there are multiple secondary tumors scattered throughout the body at unknown locations. The fact that metastases can remain occult, or hidden, for a considerable time, until they are also capable of invading and metastasizing, is especially insidious. Nowhere is the analogy of a tumor to a conspiracy more apt than in discussing tumor invasion and metastasis.

As one might imagine, the properties of local invasion and wide dissemination are linked, in that each requires a tumor cell that is able to grow and function while separated from other tumor cells. Both invasion and metastasis also require cells that are able to move, either the short distance needed for local invasion, or the long distance that may be required for wide dissemination. And neither invasion nor metastasis can occur unless a primary tumor has progressed enough to contain tumor cells with a propensity for invasion and metastasis. To understand the relationship between tumor progression and the production of cells that are capable of invasion and metastasis, we must first examine the typical pattern of tumor progression.

THE PATTERN OF TUMOR PROGRESSION

A tumor probably originates as a single transformed cell somewhere in the body, and that cell must undergo a long process of development, analogous to the development of an organ, before the cell can form a tumor. The cell undergoes countless cell divisions to form a mass that may be comprised of a billion (10^9) cells at the time of diagnosis. But tumor cells have very stringent constraints placed on them as they grow, the most stringent being that each of the newly created cells must have a steady supply of nutrients in order to keep growing.

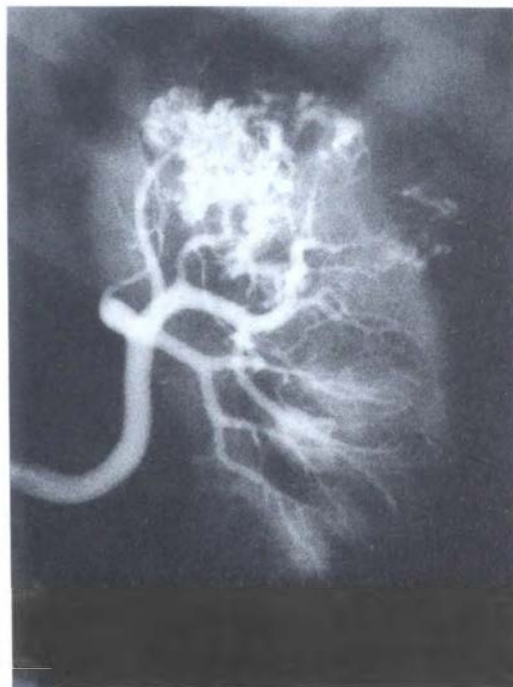
If a single cell is transformed, so that it no longer responds to those signals that regulate the growth of normal cells, it may undergo dozens of divisions before encountering any serious limitation of oxygen or nutrients. The original transformed cell was supplied with adequate nutrients for metabolism and growth because of the close proximity of blood flowing through regional capillaries. While the transformed cell may not have been directly adjacent to any of these capillaries, it was still close enough that diffusion of oxygen and nutrients through tissue could satisfy the needs of the cell. But at some point that single cell forms a mass of cells, some of which are separated from blood in the capillaries by an ever-increasing mass of tumor cells. No new vasculature has yet been formed to carry additional blood to the tumor, so that some of the tumor cells may actually experience a chronic shortage of the nutrients necessary for growth. These tumor cells may be unable to grow and, in fact, may begin to die, as substrates for cellular metabolism become harder and harder to obtain.

A tumor lacking sufficient vasculature can remain essentially dormant for years. Dormancy is forced on the tumor because cells lack sufficient nutrients to grow beyond a certain critical size, but below that size, cells are adequately supplied with nutrients. If the tumor were to be diagnosed at this point, it might be relatively easy to obtain a surgical cure, since the tumor mass would be only about a millimeter in diameter (Fig. 6). Furthermore, a tumor at this stage has not yet begun to invade surrounding tissues, and has not had a chance to metastasize. A tumor of this type is called a carcinoma *in situ* by a pathologist, although pathologists seldom see such tumors because they are asymptomatic. But occasionally tumors of this type are found by accident, during surgery undertaken for an unrelated reason.

A tumor can grow past the critical size, and leave the dormant *in situ*

phase, if and only if it is able to induce growth of new blood vessels into the tumor mass. These new vessels carry blood to the tumor cells, so that they can resume growth. Formation of new blood vessels in a tumor has been called angiogenesis; it occurs when endothelial cells, those cells that line blood vessels, are stimulated to divide and to move toward the tumor. The division rate of endothelial cells can be 20 to 2000 times faster in tumors than in normal tissues. Stimulated endothelial cells move and divide in a concerted fashion, to form miniature hollow tubes that are initially blind-ended. However, as these tubes form, they join to each other, forming functional new blood vessels. Often these new tumor blood vessels are structurally abnormal and less effective than blood vessels in normal tissues (Fig. 21), perhaps because the endothelial cells grow so rapidly, but the vessels still enable tumor cells to obtain nutrients. The fact that angiogenesis is required of all tumors able to form a mass larger than about 1 mg (10^6 cells) means that tumor growth could potentially be blocked if tumor angiogenesis were somehow inhibited. Inhibition of tumor angiogenesis is thus potentially a viable cancer therapy.

Figure 21. X-ray computed tomographic (CT) image of a large kidney tumor. The patient was infused with an X-ray contrast agent through a catheter that was inserted into an artery in the leg. Contrast agent was released into blood flowing into the kidney, to highlight blood vessels within that organ. The tumor is visible in the upper part of the kidney as a tangled web of blood vessels; such a tortuous mass of vessels is often referred to as "tumor vasculature." (Photograph courtesy of Dr. Udo Schmiedel, University of Washington School of Medicine.)



The growth rate of tumors appears to be determined by their ability to induce angiogenesis, since weakly angiogenic tumors tend to grow very slowly. Several different angiogenic factors have been identified in tumors, but angiogenic factors are also expressed in normal tissue, although under closely regulated conditions. Loss of normal regulation of angiogenesis is seen in certain diseases other than cancer, all of which are characterized by pathologic growth of blood capillaries. Diseases characterized by abnormally extensive angiogenesis include atherosclerosis, diabetic retinopathy (blindness), rheumatoid arthritis, psoriasis, and glaucoma. Diseases in which angiogenesis is abnormally slow include delayed wound healing, nonhealing bone fractures, and hemifacial microsomia, an unusual condition in which one side of the face is abnormally small and stunted. Clearly, if pathologic angiogenesis could be controlled, then victims of cancer and of these various other diseases would benefit greatly.⁴⁵

Once vascularization of the tumor has occurred, the tumor is free to grow more rapidly. Under optimal conditions, tumor cells divide continuously and can produce a large number of new cells in a short period of time. A clinically detectable tumor (1 g or 10^9 cells) may be produced in a few months or years, depending on the cell growth rate and the proportion of new cells that survive. Whether a particular newly produced cell survives or not is largely a matter of chance, but if a cell carries a mutation that makes it unusually well adapted, it may survive even though conditions prevent the growth of other tumor cells. If the mutated cell can transmit the mutation to its daughter cells, the daughter cells will also enjoy a growth advantage relative to other cells in the tumor. This process can potentially lead to the production of new clones of cells within a tumor, each clone somewhat different from each other and from the original cancer cells. A process of natural selection results, with selection producing clones of cells that are better able to grow a tumor. This has been called clonal evolution, and it can lead to the production of new cells within a tumor that have an enhanced ability to invade and metastasize (Fig. 13).

TUMOR CELL INVASION

Tumor invasion into normal tissue occurs because tumor cells do not respect the boundaries that limit normal cell growth. Epithelial cells, those cells at the surfaces of organs or structures, are anchored to a thin layer of connective tissue that supports the surface cells and prevents underlying cells from

breaching that surface. Individual epithelial cells attach to this membrane at their base, so the membrane has been called a basement membrane. Basement membrane is also found beneath the endothelial cells lining a blood vessel, the cells on the inner surface of the trachea, and the cells lining the intestine. The basement membrane remains intact in benign disease states, such as cell proliferative disorders or even carcinoma *in situ*. But the basement membrane is breached when a carcinoma *in situ* becomes a malignant tumor. For example, malignant transformation can affect epithelial cells lining the milk ducts of the breast. When transformation affects only the duct cells, without breaching the basement membrane underlying these cells, this is a carcinoma *in situ*. But breast carcinoma *in situ* can progress rapidly, and transformed epithelial cells are invasive of surrounding tissue. When the basement membrane is breached, so that malignant cells infiltrate the supportive tissue of the breast, the cells form an invasive carcinoma. Invasive tumor cells can also penetrate through the basement membrane covering an organ, which separates that organ from adjacent tissue, so that tumor cells come in contact with cells in adjacent tissues and organs.

It is important to note that invasion by a tumor cell is an active, not a passive, process. Passive movement of tumor cells through a basement membrane cannot occur, because the membrane is ordinarily quite tough and lacks pores. Furthermore, cells are normally tightly attached to basement membrane, or to the extracellular matrix that surrounds each cell. Thus, penetration of a basement membrane by a tumor cell can only occur if the cell is motile and can detach itself from the connective tissue around it, and if the cell is able to erode through the basement membrane. A tumor cell can erode both basement membrane and extracellular matrix by releasing enzymes that digest proteins. One of the major structural proteins of basement membrane is collagen, and collagen is specifically digested by an enzyme called collagenase. In a graded series of mouse melanoma cell lines of increasing metastatic ability, melanoma cell invasiveness was directly related to the amount of collagenase secreted by the cells.⁴⁶

Benign human cancers of the breast, colon, and stomach all show low levels of collagenase activity, while malignant tumors at these sites typically have high levels of collagenase activity. If collagenase activity is reduced by turning off expression of the collagenase gene, then cells lose their invasive ability. For example, in human melanoma cell lines, treatment with retinoic acid (vitamin A₁) results in a reduction of expressed levels of collagenase.⁴⁷ Retinoic acid thus inhibits the ability of melanoma cells to invade through

basement membrane, suggesting that collagenase activity is responsible for the invasive ability of these cells. However, tumor cells secrete more than a half-dozen other protein-digesting enzymes besides collagenase, including such potent enzymes as acid phosphatase and cathepsin. Experiments have suggested that several different enzymes may be required for a tumor cell to successfully invade tissue.

Tissues in the body have mechanisms that normally limit the amount of basement membrane turnover.⁴⁸ During wound repair or in various inflammatory conditions, lytic enzymes that are similar to those produced by tumor cells are released by normal macrophages. But various kinds of protease inhibitors are present in the tissue and in the bloodstream, to limit the amount of tissue degradation caused by these enzymes. For example, α_2 -macroglobulin is a potent circulating inhibitor of collagenase. Experiments have shown that treatment with collagenase inhibitors can retard invasion of tumors into mouse tissue, and can also arrest invasion of cells in culture. It is likely therefore that, in the area of a tumor, the balance between collagenase activity and anti-collagenase activity is lost, so that protein digestion predominates. This could occur because massive amounts of collagenase are secreted, or because tumor cells secrete an inhibitor of the anticollagenase, or because the metabolism of cells that secrete basement membrane is somehow disturbed by cancer cells.⁴⁹

Clonal evolution within a tumor can actually favor the acquisition of invasive behavior by a tumor cell, since invasive cells are likely to come in closer contact with established capillaries and to have greater access to blood flowing within these capillaries. Invasion of tumor cells directly into the capillaries can occur, so that the tumor cells are literally bathed in the nutrients of the bloodstream. This is clearly advantageous for the tumor cells, since they will have access to high concentrations of all metabolic substrates. Furthermore, this sort of vascular invasion can enable tumor cells to spread or metastasize to other parts of the body through the bloodstream. However, it should be noted that tumors can be invasive without being metastatic (e.g., brain tumors), although tumors cannot be metastatic without first being invasive.

Once a malignant cell begins to erode normal tissue, it can actually creep into the space it creates (Fig. 22). Tumor cell movement occurs as a series of discrete steps: first the tumor cell attaches to the basement membrane; next the membrane is dissolved in an area immediately beneath the site of attachment; then the tumor cell moves into the newly created space. The ability of tumor cells to bind to basement membrane is related to their ability to digest this

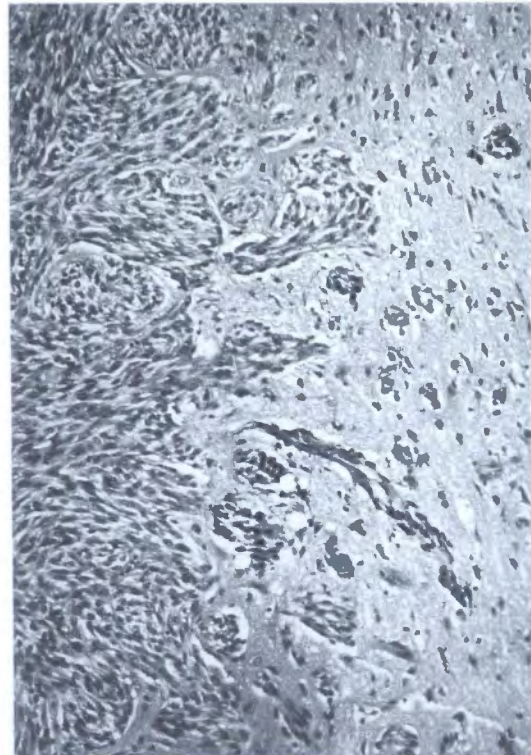


Figure 22. Microscopic view of invasive cells from a rat 9L brain tumor. Tendrils of dark-colored tumor cells are seen infiltrating into surrounding normal brain at the right of the picture.

membrane. Laminin is a component of basement membrane, and laminin receptors have been identified on a variety of different tumor cells. Laminin binding capacity is 50-fold higher in invasive human breast carcinoma cells than in normal breast cells or benign breast tumor cells. Thus, an abundance of laminin receptors on the surface of invasive tumor cells may enable those cells to bind to and digest basement membrane. After the basement membrane has been breached, tumor cells move through the membrane by extending fingers of cytoplasm that appear to pull the cell along.

Tumor cell invasion into normal tissue is not a random process or the result of a haphazard secretion of protein-digesting enzymes. The processes involved in cell invasion occur in an orderly fashion, with attachment to the matrix followed by matrix dissolution, cell detachment from the matrix, and cell movement. The cell literally pulls itself along on the matrix much as a person climbs a ladder, with a carefully orchestrated series of steps. If the matrix

around a tumor cell were simply dissolved away by a general release of proteolytic enzymes, there would be no matrix for the tumor cell to pull along. Lysis of all of the protein matrix around the tumor cell would remove the structure that supplies traction for the cell, so the cell would be unable to move. Thus, tumor cells must use proteolysis in a very organized fashion, implying that this process is important to the survival of the tumor cell.

Once the basement membrane has been breached, tumor cells are free to invade adjacent tissue. The mechanisms used by tumor cells for tissue invasion are rather poorly understood, but a combination of both mechanical and enzymatic means may be required for tumor cells to insinuate themselves into tissues. The physical pressure of a growing tumor may be able to mechanically force apart normal tissue, enabling some tumor cells to move into the gaps created. The proteases secreted by tumor cells also play a major role in facilitating tumor invasion, by digesting away the extracellular matrix surrounding normal cells. Proteases can be secreted in large amounts by tumor cells and these proteases can destroy normal tissue integrity. For example, the protease cathepsin is synthesized and secreted by many malignant human cells in culture, and cathepsin digests collagen and several other structural proteins in extracellular matrix. Analysis of enzyme expression in over 100 human cancers has shown that tumor cells express much higher levels of cathepsin L than do normal human cells. Human tissues normally express 2–7 units of cathepsin L, whereas expression of cathepsin L in human tumors can range up to 700 units, with the mean level of expression in different cancers being 6–11 units.⁵⁰ The proteases that tumor cells release can destroy normal cells as well as extracellular matrix, so these proteases can cause destruction of tissue around the tumor.

Tumor cell motility is stimulated by certain motility factors or “scatter factors,” which cause tumor cells to disperse away from the site of a primary tumor. The direction of tumor cell migration can also be determined by chemicals released by normal organs that act as attractants for the tumor cells. Such “chemoattractants” may play a role in determining the organ(s) to which a particular tumor cell can metastasize. The molecules that act as chemoattractants for tumor cells are quite diverse, but all are likely to be found in inflamed areas within a tissue. For example, collagen and the breakdown products from digestion of collagen are attractants for some tumor cells, whereas other tumor cells are attracted to substances released by the white blood cells called macrophages. Since macrophages normally congregate at sites of cell damage, inflammation, or bacterial infection, the fact that tumor cells are attracted to

substances released by macrophages means that tumor cells will tend to move toward areas where normal tissue architecture is already disrupted. Chemotactic factors appear to modulate the invasive and metastatic potential of tumor cells and may explain the tendency of some tumors to metastasize to areas of tissue injury.

TUMOR CELL METASTASIS

Overall, about 50% of all cancer patients have metastatic disease at the time of diagnosis. Metastasis is present in virtually every cancer that proves fatal, and metastasis is usually blamed for the fatal outcome of advanced tumors. Metastasis can increase the total tumor burden for the patient at a very rapid rate, and each metastatic tumor is capable of further mutation, to produce new clones of invasive and metastatic tumor cells. In addition, metastatic tumors are each capable of destroying normal tissue, obstructing vital passageways within the body, or having direct hormonal effects on the general health of the cancer patient. Metastases are especially insidious because, even though the physician may know that there are numerous metastases in a particular patient, it is often impossible to pinpoint where those secondary tumors are until they become capable of seeding additional metastatic tumors. In many cases it is possible to obtain a surgical cure of a primary tumor, but the joy of this event must be tempered by knowledge that there may be occult metastases in the patient. It is quite likely that, long after cancer therapy has improved to the point where cure of a primary tumor is routine, problems with identifying and treating metastases will remain.

All tumors that are metastatic are first invasive, since metastasis cannot occur unless the tumor cells gain access to some passageway within the body through which to spread. Metastasis most commonly occurs through the circulatory system or through the lymphatic system. The latter is a system of vessels that carry lymph, a clear yellowish fluid, as it drains from tissues. Lymph is comprised of the fluid that moves out of the blood vessels, and through or around cells, transporting cell waste as it goes. Lymph fluid is collected into small vessels similar to capillaries, after which it moves to larger vessels and finally drains into lymph nodes. Lymph vessels are not bounded by a basement membrane like blood vessels are, so lymph vessels are often the path of least resistance for a metastatic tumor cell. This could account for why lymph node involvement is so frequently the first type of metastasis seen in

patients. Tumor cells can be shed into the lymphatic system and can move to lymph nodes, where cells lodge or are filtered out. Since lymph fluid is rich in various substrates for cell growth, tumor cells that lodge in a lymph node are potentially able to keep growing there. Ultimately, lymph fluid is drained back into the bloodstream, so some tumor cells may actually gain access to the bloodstream by moving through the lymphatic system.

Generally, there is a correlation between the ability of a tumor to induce angiogenesis and the ability of that tumor to metastasize. This is because blood vessels are often the highway by which tumor cells move. However, occasionally it is not necessary for cells to gain access to either the vasculature or the lymphatic system in order to metastasize. For example, brain tumors can shed cells into the ventricles or cavities within the brain, so that tumor cells can move through the ventricles to other sites within the brain. Liver cancers can release malignant cells into the fluid of the abdominal cavity, so that tumor cells have access to other organs within the abdomen and can establish metastatic colonies on various organ surfaces. Ovarian cancers also frequently seed metastases by releasing tumor cells into the abdominal cavity.

Metastatic cells generally begin to metastasize by invading through the basement membrane of a blood capillary and into the lumen of that capillary (Fig. 23). If the tumor cell instead invades into a lymph vessel, invasion is easier because lymph vessels lack a basement membrane. In any case, once the tumor cell has gained access to the lumen of the vessel, the cell passively glides through the vessel as the blood or lymph fluid surrounding the cell flows along. The tumor cell can be carried a considerable distance through the bloodstream, but eventually the cell will pass through the heart and into a second capillary bed. There the tumor cell may lodge, as the diameter of the capillary lumen narrows. If the tumor cell is capable of binding to the basement membrane of the capillary in the new location, then the cell may be able to penetrate through the capillary wall and into the surrounding tissue. This process, in which a tumor cell moves through the wall of a capillary and into surrounding tissue, is called extravasation. After extravasation, tumor cells can invade into the adjacent tissue and form a second tumor.

The whole process of tumor metastasis has been observed experimentally in a rabbit ear, using a very clever technique. The ear of the rabbit was first fitted with a small glass chamber that sandwiched the ear tissue so that it could be held motionless under a microscope. This permitted scientists to observe as tumor cells were injected into the bloodstream near the ear chamber. Tumor

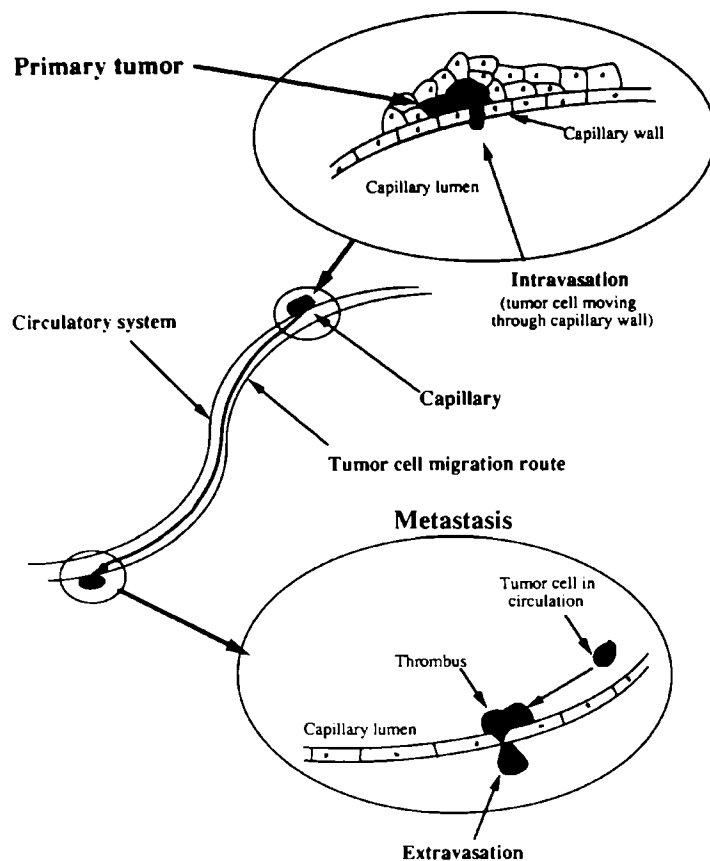


Figure 23. Summary diagram of metastasis, showing intravasation of a tumor cell into the capillary vessel lumen, the migration of tumor cells through the circulatory system, and the extravasation of tumor cells in a second capillary bed.

cells flowed with the blood into an area observed under the microscope, then adhered to the walls of the capillary. This occurred even though blood was still flowing quite rapidly past the tumor cells. Within 30 minutes a blood clot formed around the tumor cells, and within 24 hours tumor cells had begun to divide within the capillary lumen. By 48 hours tumor cells began to invade through the capillary wall, and by 72 hours a tumor metastasis was established at the site of extravasation.⁴⁸

The mere presence of tumor cells in the bloodstream does not guarantee that metastases will form. The vast majority of tumor cells shed into the

bloodstream do not survive in the blood, and many tumor cells that arrest in a capillary are unable to extravasate. It has been estimated that fewer than 0.1% of tumor cells in the bloodstream survive long enough to form distant metastases. However, although the proportion of successful metastatic cells is very low, the number of cells shed into the bloodstream can potentially be very high. Recall that the cell loss rate in human tumors is thought to be high, since there is often a very large discrepancy between the calculated rate of cell growth and the measured rate of tumor growth. Mean cell loss rate from human tumors may be as high as 45%, meaning that 90% of newly produced tumor cells are lost from the tumor. Tumor cells can be lost from the tumor in many ways (e.g., death of cells unable to tolerate the environment within a tumor, inability of genetically damaged tumor cells to survive, or attack on the tumor cells by host defenses), but probably many tumor cells are lost from tumors through metastasis.

As tumor cells move through the circulatory system and arrest in the capillaries, the great majority of tumor cells die. Surviving tumor cells presumably are successful because some aspect of their cellular physiology makes them better able to tolerate the harsh conditions they encounter in the new location. Thus, there is a continual process of natural selection going on, with tumor cells surviving only if they are able to extravasate and form a new tumor. The result of this selection is that tumor metastases generally contain cells that are more aggressive and more metastatic than were cells in the primary tumor. This has been clearly shown in an experiment in which melanoma cells were harvested from the skin of a mouse, and injected into the tail vein of a second mouse.⁵¹ After about 3 weeks, the second mouse was sacrificed and dissected, to determine the number of metastatic tumors in the lungs and to harvest cells from those tumors. Cells from a lung metastasis were harvested and injected into the tail vein of a third mouse. After another period of time, the third mouse was also sacrificed, and cells were again harvested from metastatic lesions in the lungs and injected into another mouse. After this process had been repeated many times, it was found that the number of lung metastases produced by the melanoma cells increased over time. This is because melanoma cells were selected for their ability to metastasize, since only metastatic tumor cells were harvested and injected into another mouse. Each time this selection was applied, the resulting cells increased in metastatic ability. Although this experiment was done in mice, it is clear that a similar process can potentially occur in a patient over time.

METASTASIS AS A SELECTIVE PROCESS

The description of metastasis given above implies that tumor cells are able to metastasize equally well to every capillary bed in the body. This is not true, since tumor cells exhibit a fair degree of selectivity as to the organ to which they will metastasize. Most cancers will metastasize to regional lymph nodes and to the liver and lung, but beyond that, the pattern of metastasis becomes much more idiosyncratic. Odd patterns of metastasis are seen, as in the propensity of prostate cancers to metastasize selectively to bones of the lower spine. Sometimes different tumors will metastasize to the same organs, but one tumor type will have a wider pattern of metastasis than the other. For example, kidney cancers frequently metastasize to lung, liver, and bone. Lung cancers metastasize to liver and bone, but they also frequently spread to regional lymph nodes, brain, kidney, adrenal, thyroid, and spleen. It is not known why kidney cancers partially overlap with lung cancers, in terms of the organs to which they metastasize, but will seldom spread to brain, adrenal, thyroid, or spleen. Odd patterns of nonreciprocal metastasis can also be seen. For example, breast cancers often spread to the ovary, but ovarian cancers seldom spread to the breast. Many different cancers seed metastases to the brain, but a brain tumor almost never spreads to any location outside the nervous system.

The basis for the selective spread of tumor metastases is poorly understood, but some of the selectivity can be explained by considering the tissues to which tumor cells have access. This means simply that tumor cells are more likely to metastasize to places that they can easily reach. The lungs are a very common site of metastasis, perhaps because all of the blood in the body goes through the lungs every time it makes a complete circuit through the body. Similarly, liver tumor cells shed into the abdominal cavity will frequently form metastases on the surfaces of nearby organs, but will be unable to form metastases at sites deep within the same organs. Prostate tumor cells may spread to the bones of the lower spine because the prostate and the lower spine are both drained by veins that join together on the way to the larger veins of the lower abdomen. Studies have shown that tumor cells injected into animals will often lodge and form tumors in the first organ that they encounter as they circulate. Furthermore, clinical experience indicates that tumors frequently metastasize to the first organ that would be encountered by cells shed from the primary tumor. Thus, metastasis can be a function of simple mechanical entrapment of tumor cells in the capillary bed of an organ. However, there is

often no correlation between the site of initial arrest of a tumor cell and the subsequent pattern of metastatic tumor growth.

Another explanation for the selective nature of tumor metastasis, called the “seed and soil” hypothesis, was first suggested more than a century ago. This hypothesis suggests that growth of a metastasis is very much like the growth of a plant from a seed: a plant cannot grow unless the seed is capable of germinating under the particular soil conditions that it encounters. Many seeds are sown in soil that cannot support growth, and these seeds fail to germinate. Thus, there is an interaction postulated between a metastatic cell and the tissue into which it metastasizes. If tissue conditions permit tumor cell growth, then a metastasis may well develop, but a metastasis cannot develop in the absence of a metastatic cell and a permissive tissue environment. The fact that different metastatic patterns are seen for different tumors suggests that the metastatic spread of cancer cells is not a random event, or simply a function of tumor access to tissue. Rather, it may be a reflection of specific properties of tumor cells and host tissues.

Strong evidence in support of the “seed and soil” hypothesis was provided in the experiment discussed previously, in which melanoma cells were selected for their ability to metastasize to mouse lung. When these cells are injected into the tail vein of a mouse, the cells tend to form metastatic colonies in mouse lung. This should not be too surprising since blood returning from the tail vein of a mouse returns first to the heart and then goes directly to the lungs. Thus, capillaries in the lung are the first ones encountered by injected mouse melanoma cells and it is to be expected that tumor cells would arrest and form metastases in the lungs. However, if tumor cells are injected into the blood leaving the heart through the aorta, this blood must first go through capillaries elsewhere in the body before the tumor cells return to the heart and go to the lungs. The expectation would then be that tumor cells injected into aortic blood would form metastases primarily in tissues outside the lungs, since these tumor cells would arrest in capillaries before they ever reached the lungs. However, when the experiment is actually done, tumor cells injected into aortic blood still form metastatic tumors primarily in the lungs.

The discrepancy between metastatic spread of a tumor and vascular delivery of tumor cells implies that there is a type of “homing” of tumor cells to an organ. Localization of tumor cells to a specific organ could result from the adhesion of metastatic tumor cells to a particular type of cell in that organ. Since metastatic tumor cells first encounter endothelial cells lining the capillaries of an organ, perhaps there are differences in the endothelial cells of

different organs that cue a metastatic tumor cell as to when it has homed to an appropriate organ. If mouse melanoma cells are exposed to thin slices of mouse tissue, the tumor cells stick more tightly to slices of lung than they do to slices of liver, brain, heart, or other organ. If a lymphoma cell line that preferentially metastasizes to liver is placed in culture with cultured liver cells, tumor cells clump together with liver cells, indicating that the two cell types are binding to one another. Thus, the distribution of metastatic tumor cells in a mouse tends to reflect the pattern of binding of tumor cells to different mouse tissues in culture.

GENE MUTATIONS AND TUMOR METASTATIC ABILITY

There is good evidence that development of metastatic ability by tumor cells is a result of genetic instability, combined with natural selection for those cells with enhanced invasive or metastatic ability. The relationship between genetic instability and metastatic ability is shown by the fact that metastatic cells are more likely to undergo mutation in culture than are nonmetastatic cells. In fact, metastatic cells brought into culture are quite likely to mutate into a form that is either more metastatic than the parent cells or less metastatic. Therefore, metastatic behavior of tumor cells is highly unstable. Highly metastatic clones of tumor cells have been shown to have a fivefold higher mutation rate than nonmetastatic clones of cells, when both clones are exposed to some mutagenic chemical. The enhanced mutation rate of highly metastatic cells suggests that these cells may have a greater ability to adapt to a new environment than nonmetastatic cells. If metastatic cells are more likely to mutate, then metastatic tumors are more likely to be highly heterogeneous. This is important because tumor heterogeneity has been implicated as a major factor leading to tumor resistance to therapy. Finally, if metastatic cells are genetically unlike the primary tumor, it may be difficult or impossible to make antibodies to a primary tumor and then use those antibodies to reveal the presence of occult metastases.

Tumor cell metastasis may be related to the expression of certain cellular oncogenes. If the mouse cell line 3T3 is injected into the skin of a mouse, those cells cannot induce formation of a tumor. However, if a mutated form of the cellular oncogene *ras* is introduced into 3T3 cells before they are injected into mouse skin, tumors are produced that are metastatic and quite aggressive.⁴⁹ Several other activated cellular oncogenes are also capable of increasing the

metastatic ability of 3T3 cells, suggesting that the metastatic ability of tumor cells is influenced by cellular oncogenes.

Tumor suppressor genes may also play a role in modulating the metastatic ability of tumor cells. A gene called p53 suppresses tumor growth, and loss or mutation of this gene is associated with progression of many different tumors from the benign to the malignant state. Mutant p53 has been found in cancers of the skin, lung, breast, and colon. A large number of melanomas from different stages of tumor progression were tested for expression of mutant p53, and an increased incidence of p53 mutation was found in metastatic melanoma, compared with primary melanoma.⁵² Precancerous skin lesions called dysplastic nevi showed p53 mutations in no cases (0 of 3). In small primary melanomas, 3 of 5 tumors (60%) showed p53 mutations, while in large primary melanomas (> 1.5 mm) 11 of 15 tumors (73%) showed p53 mutations. Examination of melanoma metastases showed that 94% of the metastatic tumors (31 of 33) had p53 mutations. Thus, mutation of the p53 gene is associated with melanoma metastasis.

Recently another gene, called nm23, was specifically linked to the metastatic ability of tumor cells. This gene was expressed in reduced amounts in several highly metastatic melanoma cell lines.⁴⁹ Transfection of the nm23 gene into highly metastatic melanoma cell lines decreased their ability to metastasize. The nm23 gene is expressed in nonmetastatic breast cancers, but is mutated or lost in metastatic breast tumors. Reduced nm23 expression in breast cancer is associated with the presence of lymph node metastases, as well as with decreased disease-free survival time and decreased overall patient survival time. However, in colorectal cancer, no association was found between nm23 expression and the rate of tumor metastasis. It is believed that the nm23 gene contributes to normal development of tissues, perhaps by affecting cell-to-cell communication. Loss of nm23 would thus lead to a disruption of cell-to-cell communication, and a disordered tissue state that might favor metastatic tumor progression.

OTHER FACTORS THAT AFFECT TUMOR METASTATIC ABILITY

The ability of a tumor to metastasize to a particular tissue can be affected by factors other than the simple ability of a tumor cell to bind to a particular tissue. A hormone called parathyroid hormone-related protein (PHrp) was found in about 60% of a series of primary breast cancers. Breast tumors are well

known for their ability to metastasize to sites in bone, and the ability of PHrp to induce bone resorption is also known. PHrp expression was found in 92% (12 of 13) of breast cancer metastases in bone, but in only 17% (3 of 18) of metastases in nonbone sites. Thus, a bone-resorbing factor was specifically secreted by tumors with a propensity to metastasize to bone. This suggests that PHrp expression may contribute to the ability of breast cancers to metastasize to bone.⁵³

Recent experiments suggest that treatment of a primary tumor can actually increase the metastatic ability of surviving tumor cells. Furthermore, treatment of a primary tumor can have an effect on the growth of established metastases at a distance from the treated primary tumor. The proportion of tumor cells undergoing cell division was measured in metastatic tumors, to determine the growth rate of the metastases. An increase was found in the growth rate of metastases after surgery to remove the primary tumor. Stimulation of metastatic tumor growth rate occurred following surgical removal of the primary tumor in each of six different tumor lines, and the increased growth rate was not caused by trauma during the operation.⁵⁴ However, preoperative administration of therapy (cyclophosphamide, tamoxifen, or radiation) prevented the increase in growth rate of the metastases after removal of the primary tumor. It was proposed that a growth-stimulating factor was present in the bloodstream following primary tumor removal in mice. If the same thing happens in patients, this could have important implications for the timing of adjuvant therapy relative to "curative" surgery.

CHAPTER 7

Physiological Interactions between Patient and Tumor

The actual growth rate achieved by a tumor is a compromise between the maximum rate of tumor growth possible under optimal conditions, and the suboptimal conditions that the tumor actually encounters. Conditions that favor tumor growth can be intrinsic to either the patient or the tumor, while conditions that oppose tumor growth are largely inherent to the patient. Therefore, tumor progression can be thought of as an ongoing interaction between cancer patient and tumor. Controls that the patient exerts on tumor growth are complex and poorly understood, and controls that the tumor exerts on the patient are only somewhat better known.

PATIENT EFFECTS ON THE TUMOR

There are many subtle interactions possible between patient and tumor, some of which could potentially enhance the growth rate of the tumor. Although direct stimulatory effects of the patient on tumor growth rate are not the rule, they do occur (e.g., estrogen-stimulated growth of breast tumors, testosterone-stimulated growth of prostate tumors). Such stimulation is always potentially possible because of the mixed bag of growth factors normally present in the bloodstream. Tumor cells typically have a reduced requirement for growth factors when compared with normal cells, if both are grown in culture. But this does not mean that tumor cells are free of dependence on host growth factors. In fact, the results from work with cultured tumor cells could potentially be very misleading. When tumor cells are placed in culture, culture conditions may inadvertently select for those cells with a reduced requirement

for growth factors. This is because the process of bringing cells into culture gives a natural advantage to those cells that are best able to tolerate the growth conditions employed. Those tumor cells that have few growth requirements in culture enjoy a growth advantage, and may overgrow and replace those cells that are still dependent on growth factors. After a period of growth in culture, the only cells surviving may be those that are free of dependence on growth factors. Thus, bringing tumor cells into culture can potentially select out a fraction of cells that are not truly representative of the tumor mass.

Human small-cell lung carcinoma (SCLC) is a particularly aggressive lung cancer that grows rapidly in the patient. Yet SCLC cells have such stringent requirements for growth factors that they are unable to grow in culture unless the necessary growth factors are supplied. At first SCLC cells could only be grown in culture if conditioned medium was used. Conditioned medium is simply growth medium that has already been used once for culturing cells, so that the medium contains any growth factors released by the previous cells. Growth of SCLC cells in conditioned medium implies either that SCLC cells have certain growth factor requirements that were not met under normal culture conditions, or that there is some inhibitor of cell growth that was present under normal culture conditions.

An ambitious project was undertaken to determine the nutrients and growth factors that enable SCLC cells to grow in culture.⁵⁵ Five ingredients were found to be required for growth of SCLC cells in culture: selenium, hydrocortisone, insulin, transferrin, and estradiol. Selenium is a nonmetallic chemical element of unknown importance to growing cells. However, the other four ingredients that stimulate SCLC growth in culture are all hormones or growth factors that are potentially present in the bloodstream of a lung cancer patient. When these ingredients are all present in culture medium, SCLC cells grow and divide at a high rate for extended periods of time. When individual components of this mixture are deleted, cell growth is immediately and dramatically reduced.

Interestingly, the same study that showed that SCLC cells in culture are stimulated to grow by some growth factors, also showed that SCLC growth in culture could be inhibited by other factors. In fact, several hormones that are often present in cancer patients strongly suppressed SCLC cell growth in culture (e.g., epidermal growth factor and luteinizing hormone-releasing factor). This work has shown that tumor cells in culture can have stringent requirements for growth factors, and it is reasonable to suppose that these cells would have similar growth factor requirements if they were to form a tumor in a

patient. Therefore, the presence of growth factors in the bloodstream may facilitate tumor growth in the patient.

TUMOR EFFECTS ON THE TUMOR

A tumor can have numerous effects that depress the ability of the patient's immune system to combat the tumor. Thus, a tumor could have an indirect stimulatory effect on its own growth. This indirect modulation would be difficult to detect and even more difficult to prove. But a tumor could also conceivably have a direct stimulatory effect on its own growth. For example, it has been suggested that certain cancer cells produce and secrete self-stimulatory growth factors. These growth factors are released by one tumor cell, and then interact with receptors on the surface of nearby tumor cells (Fig. 24). Nearby tumor cells would then be stimulated to grow and divide by this growth factor. When SCLC cells are raised in cell culture, they synthesize and release a protein called bombesin-like peptide.⁵⁶ This protein can be harvested from the culture medium in which SCLC cells grow; when it is added to SCLC cell cultures it stimulates growth of these cells, but it does not stimulate growth of other types of cultured tumor cells. SCLC cells have a receptor on the cell surface that selectively binds the peptide, suggesting that only tumor cells with this particular receptor can respond to bombesin-like peptide.

These findings suggest that bombesin-like peptide acts on SCLC cells in a kind of self-stimulatory or autocrine loop. When experiments were undertaken in living rats to test this hypothesis, some potentially important results were obtained.⁵⁶ Researchers tried to interrupt the proposed autocrine loop, using an antibody that binds bombesin-like peptide. The antibody blocked binding of bombesin-like peptide to the receptor on the SCLC cell surface, and thereby inhibited SCLC growth in culture and slowed growth of SCLC tumors in rats. Thus, at some point in the future, it may become possible to block tumor growth by injecting a patient with an antibody to an auto-stimulatory protein (Fig. 24). However, this sort of clinical application is a long way off.

TUMOR EFFECTS ON THE PATIENT: PAIN

Pain is frequently associated with cancer, and it can be unrelenting and difficult to palliate. Pain can be the result of (1) invasion or destruction of

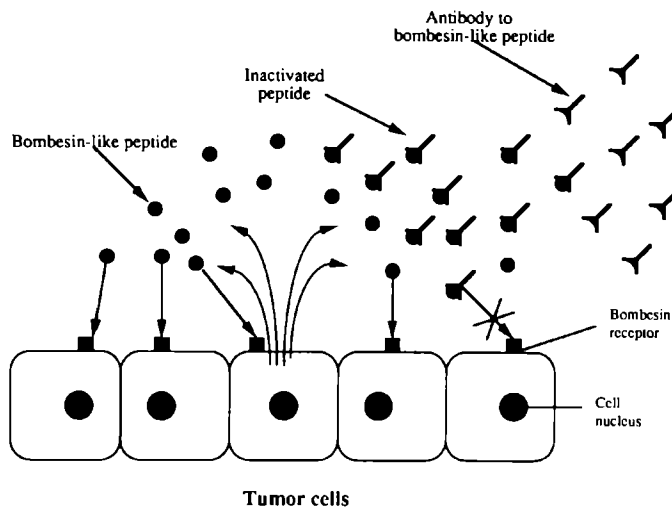


Figure 24. Diagram of an autocrine loop involving bombesin-like peptide (also called vasoactive intestinal peptide or VIP) in small-cell lung carcinoma. Some transformed cells release bombesin-like peptides (solid circles), which have a growth-stimulatory effect on surrounding tumor cells. However, antibody to the bombesin-like peptide can be administered therapeutically, so that free bombesin-like peptides are bound to the antibody and can no longer stimulate cell growth. This can actually slow tumor growth in rats, but the utility of this therapy is unproven in humans.

normal tissue by the tumor, (2) stretching of internal tissues by tumor growth, (3) pressure on an organ, (4) obstruction of a passageway, or (5) infection associated with cancer. An example of pain resulting from tissue destruction is the bone pain that often results from metastatic breast cancer. This pain is caused by irritation of the membrane adjacent to the bone, pressure on spaces within the bone, or fractures that result from bone weakening.

Pain can result from growth of tumors in an area where tissue expansion is limited. Brain tumors typically obstruct the flow of fluid through the ventricles of the brain or cause distension of parts of the brain, and this can cause severe pain. In this case the pain can be “referred pain,” meaning that pain is felt in a part of the body other than the head. When tumor growth causes the stretching of an organ, this can also be very painful. However, the growth of tumors in the abdomen is typically so slow that stretching is very gradual and there may be no associated pain. For this reason, many cancers of the stomach, colon, and liver remain occult until they are well advanced. Pain from internal organs can also cause referred pain: pain from a tumor in the esophagus is sometimes felt in the shoulder; and stomach pain may be referred to the chest rather than the

abdomen. Obstruction of a hollow organ such as the stomach, small intestine, bile duct, or colon can produce cramps and a colicky pain that may be severe if obstruction is complete.

Pain is often caused by secondary infection in the cancer patient. Infection in the cancer patient is commonplace, because of an apparent decrease in immunity to infection, and because open sores or poor tissue drainage may result from tumor growth. An infection that strikes many cancer patients is caused by varicella-zoster virus (VZV). VZV causes two distinct clinical diseases: varicella or chickenpox, a relatively benign infection of childhood; and herpes zoster or shingles, a blistering skin rash associated with severe pain. Herpes zoster apparently results from reactivation of an earlier VZV infection. The mechanism that causes viral reactivation is unknown, but VZV is apparently able to remain dormant in the nervous system for years after the initial infection. Herpes zoster is rarely fatal, even in a severely immunocompromised cancer patient, but the rash can persist for weeks, and many patients report pain months after resolution of the rash.

Certain cancers produce very characteristic patterns of pain. For example, advanced breast cancers are painful if they invade the chest wall, if they cause an inflammation of the breast, or if there is extensive swelling of regional lymph nodes. Lung cancers are often asymptomatic until there is local invasion or distant metastasis, at which time pain may become a problem. Pain in lung cancer patients is often manifested as severe shoulder or arm pain, or there may be bone pain resulting from stretching of the membranes adjacent to bone.

ANGIOGENESIS: THE INDUCTION OF NORMAL CELL GROWTH BY A TUMOR

It is frightening for the cancer patient to realize that a tumor is able to subvert the normal processes of the human body in order to satisfy the tumor's own needs. A tumor, like any other tissue, has metabolic requirements for glucose and other metabolites, and these needs can only be met by robbing nutrients from the bloodstream of the patient. In order to obtain these nutrients, the tumor must induce the growth of new blood vessels into the tumor. This process of angiogenesis occurs when endothelial cells, which line normal blood vessels, are induced to begin growing, often at a rate 20 to 2000 times faster than normal. Endothelial cells divide and move into the tumor in a concerted fashion, forming hollow tubes that join and anastomose to each other, to form

new functional blood vessels. The presence of new blood vessels permits an increase in tumor perfusion, which is defined as the amount of blood flowing through the tumor per unit time.

If a tumor fails to induce angiogenesis, or if the tumor initially grows more rapidly than the vasculature supplying it, then tumor growth will eventually slow or cease. This is because continued cell growth cannot occur in the absence of metabolic substrates for the tumor cells. Furthermore, if the tumor is already a fairly large mass, then those cells that receive an inadequate supply of metabolites will die. Yet tumor cells are remarkably tolerant of harsh conditions, so even if most tumor cells are inadequately perfused and die, some cells will be sufficiently well perfused that they can survive and continue to grow.

Several factors can combine to produce a tumor that is poorly perfused with respect to the normal tissue from which it grew (Fig. 25). Tumor blood vessels are often less effective than normal blood vessels because the tumor vessels are structurally abnormal. The abnormal form may relate to the fact that

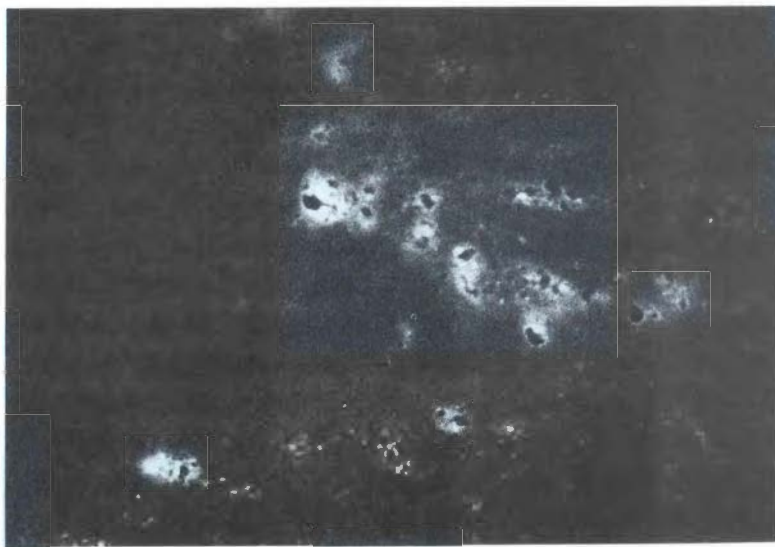


Figure 25. Photograph of a 9L rat brain tumor cut in cross section and viewed by ultraviolet light. A fluorescent dye was infused into a rat artery, the rat was sacrificed 60 seconds later, and the tumor was excised and prepared for microscopy. Because the tissue is illuminated by ultraviolet light, the dye in the vasculature fluoresces, but the tumor surrounding the blood vessels appears black. A number of blood vessels within the tumor mass contain the dye, but there are large expanses of tumor (to the upper left) that lack any fluorescence. This suggests that part of the tumor was not perfused during the 60 seconds between dye injection and rat sacrifice. Thus, there are probably many cells within the tumor mass that are hypoxic.

the endothelial cells are induced to grow so much more rapidly than normal. In fact, many of the new blood vessels are actually blind-ended sacs or tubes that open into cavities within the tumor. Once tumor blood vessels are established, other problems arise that can also cause blood flow through tumor vessels to be low. Tumor vessels are leakier than normal vessels, so fluid from the blood leaks into the tumor, resulting in water retention or edema within the tumor. Edema fluid is poorly drained from tumors, so that it can accumulate and raise the fluid pressure within the tumor. If fluid pressure increases, there will be a bulk flow of fluid away from the tumor that can oppose the movement of blood into the tumor. Recent measurements of fluid pressure in human tumors have shown that fluid pressure within a tumor may be elevated more than tenfold compared with normal tissues.⁵⁷ This elevation of pressure may mean that some parts of these tumors are not perfused at all, so cells will become hypoxic.

TUMOR PERFUSION AND TUMOR RESISTANCE TO THERAPY

Tumors are usually more poorly perfused than the normal tissue from which they are derived. For example, normal blood flow through the liver occurs at a rate of roughly 100 ml of blood per 100 g of liver tissue per min. Liver carcinoma is perfused at a rate as much as 90% lower. Normal brain is perfused at a high and very uniform rate (about 60 ml/100 g per min), whereas perfusion of brain tumors tends to be lower and more variable. In fact, brain tumor perfusion varies between 1 and 100 ml/100 g per min. Breast cancer perfusion also spans a very wide range (6 to 40 ml/100 g per min), from a low rate typical of post menopausal breast, to a rate approaching the perfusion rate of lactating breast.⁵⁸

As a rule, well-perfused tumors tend to have relatively little necrosis within the tumor mass, whereas poorly perfused tumors can have a substantial fraction of dead cells within the tumor mass. In very large tumors, the tumor may form a structure like an orange, in which the pulp of the orange is the poorly perfused, necrotic portion of the tumor, while the rind of the orange is the well-perfused, actively growing portion of the tumor (Fig. 26). In tumors that are poorly perfused, or in which there is a great deal of variability in perfusion, hypoxia can develop. Hypoxia is a state in which cells lack sufficient oxygen to maintain normal metabolism. Normal mammalian cells, when subjected to hypoxia, usually die rather rapidly. For example, a heart attack occurs when perfusion of part of the heart stops, so that some heart cells

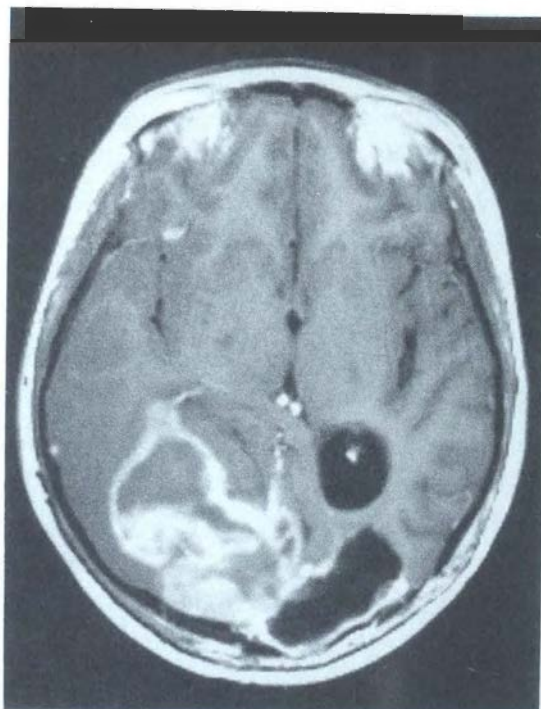


Figure 26. A large brain tumor showing heterogeneity of blood perfusion. A pediatric patient (11 years old), who had previously received surgery for glioblastoma multiforme, was examined for tumor recurrence 4 months after first surgery. This is a magnetic resonance image (MRI), obtained 10–20 minutes after injection of a contrast agent into the bloodstream. The region of brain from which tumor was previously excised is seen (lower right) as two dark cavities. The opposite cerebral hemisphere (lower left) has a very large invasive tumor originating from the earlier site of surgery. The tumor appears bright at the margins, where perfusion is relatively high, and dark at the core, where perfusion is relatively low. The tumor margin appears brighter than surrounding brain partially because perfusion is high, but also because contrast agent leaks across the blood-brain barrier at the tumor margin and accumulates, whereas

it does not accumulate in normal brain. (Photograph courtesy of Dr. June Taylor, Dr. W. Eugene Reddick, and Dr. James Langston, St. Jude Children's Research Hospital.)

become hypoxic and die. Similarly, strokes result from hypoxic cell death of nerve cells in the brain. Generally tumor cells are more resistant to hypoxia than normal cells, and tumor cells can survive for many hours in the absence of significant perfusion.

If tumor cells become hypoxic, they can become inherently more resistant to certain forms of therapy. In order for chemotherapeutic drugs to kill tumor cells, the drugs have to reach the tumor cells, usually via the bloodstream. If a tumor is poorly perfused, there may be tumor cells within the tumor mass that are never exposed to chemotherapy, even if very high levels of drug are present in the bloodstream. Furthermore, radiation therapy kills tumor cells more effectively when cells are well oxygenated, because much of the cell-killing effect of radiation is caused by production of a toxic form of oxygen called singlet oxygen. If no oxygen is present, then singlet oxygen cannot be formed.

Without singlet oxygen the number of tumor cells killed by radiation is reduced roughly threefold, compared with the number of cells killed in an aerobic tissue receiving the same amount of radiation.

If tumor cells become hypoxic, they may also be more likely to mutate. Some recent, very controversial evidence suggests that the mutation rate of cancer cells in culture is enhanced if the cells are maintained under hypoxic conditions. If the mutation rate is enhanced even a tiny amount, this can have a very deleterious effect on the patient. For example, assume that the mutation rate of hypoxic cells is increased by 1 part in 10 million. A tumor mass is usually composed of at least 100 million cells at the time of diagnosis, meaning that there may already be 10 (or more) mutant cells in the tumor. While this does not sound significant, in fact it is very important. Each of these mutant tumor cells can divide and produce progeny, so that the number of mutant cells increases. At the same time, the patient may be given therapy that succeeds in killing a certain fraction of tumor cells. If mutant tumor cells are protected from this therapy, either because they are hypoxic or because the mutation makes them more resistant to therapy, then mutant cells may survive while other tumor cells are killed. This will mean that the relative proportion of mutant cells in the tumor will increase, since therapy merely "thins the herd" of competing tumor cells.

The increased mutation rate of tumor cells within a tumor mass can lead to a sort of clonal evolution, whereby unfit (or therapy-sensitive) tumor cells are constantly being killed. This will leave behind only those tumor cells that are most fit or most resistant to therapy. Clearly, if the strongest or most resistant tumor cells are selected for by a therapy, this can lead to a very poor prognosis for the cancer patient.

EFFECT OF TUMOR PRODUCTS ON NORMAL CELLS

Tumors are perfused with blood from the general circulation, so if the tumor adds or removes substances from this blood, it can potentially have an effect on cells in the rest of the body. A tumor can have profound biological effects on nearby normal cells, as we have seen in the example of endothelial cells induced to grow into a tumor. But tumor cells can also affect normal tissues other than endothelial cells. Advanced breast cancers frequently spread or metastasize to bone, as well as to brain and liver. A recent study found what may be an underlying biological reason for this site-selectivity.⁵⁹ A particular

protein, called parathyroid hormone-related protein (PHrp), is thought to be responsible for the increased bone resorption and reduced calcium excretion that often accompany malignancy. An examination of tumor samples removed from patients at surgery showed that about 60% of breast tumors (from 101 patients) expressed PHrp. Since the protein causes bone resorption, a breast tumor that secretes PHrp might have a tendency to metastasize to bone. Expression of PHrp was also tested for in metastatic breast tumors surgically removed from various bone and nonbone sites. PHrp was found to be expressed in 92% (12 of 13) of breast cancer metastases in bone, but in only 17% (3 of 18) of breast cancer metastases in nonbone sites. This shows that tumor cells that produce a bone-resorbing agent may be better able to invade bone,⁵⁹ and also that tumor products can have profound effects on normal cells.

Prostate cancer is a very common cancer in older men, and tends to metastasize to bone. Metastatic tumors in bone usually show an abnormal local increase in bone formation, suggesting that prostate cancer cells may directly affect bone cell metabolism. Recent work has found that when prostate cancer cells isolated from a patient are grown in culture, the cells secrete a protein that stimulates human bone cells to grow two- to tenfold more rapidly than normal.⁶⁰ Cultured prostate cells secrete several proteins that resemble normal human growth factors (including insulin-like growth factor, basic fibroblast growth factor, and transforming growth factor β , as well as a previously undescribed growth factor that specifically stimulates bone cells). Production of these various growth factors probably plays a role in the intense stimulation of bone growth that occurs at sites of prostate cancer metastasis.

HORMONE SECRETION BY THE TUMOR AND EFFECTS ON THE PATIENT

Cancer cells can secrete a fairly broad range of hormones or hormone-like molecules that can have a potent effect on tissues remote from the cancer, and may even affect the general health of the cancer patient. Hormones are powerful bioactive molecules that are normally secreted in tiny quantities by a limited number of tissues or organs in the body. Hormone synthesis and release is very tightly regulated under normal conditions, because these molecules have such a strong effect on the physiology and health of the individual. Inappropriate hormone secretion, either hormone deficiency or hormone excess, is associated with a range of very serious medical conditions (including diabetes, dwarfism,

Cushing's disease, hyperthyroidism, acromegaly, failure to reach sexual maturity, and numerous other more obscure medical conditions). Because hormones are such potent molecules and can elicit a very complex web of responses in tissues throughout the body, inappropriate release of hormones is a serious, potentially lethal, condition. In fact, any production of hormones by tumors is inappropriate, even if the tissue where the tumor originated normally secretes hormones, because hormone production by tumors occurs in an unregulated, even random, manner.

Ectopic hormone production by a tumor is defined as production of a hormone that is not made by the normal tissue from which the tumor is derived. A diagnosis of ectopic hormone production in a given patient can be difficult to make, because hormone levels in the body are usually at very low levels. However, clinical evidence for ectopic hormone production can include the following⁶¹:

1. Association of a tumor with elevated levels of a hormone in the blood
2. Regression of clinical signs of hormone excess after tumor surgery
3. Persistent clinical signs of hormone excess after surgery to remove the organ normally responsible for hormone production
4. Demonstration of hormone excess within the tumor itself
5. Direct demonstration of hormone synthesis by the tumor

New, highly sensitive assays for hormone production have shown that many tumors produce ectopic hormones. In fact, biochemical evidence suggests that ectopic hormone production is much more common than indicated by clinical evidence alone. This may be because a tumor can release inactive (or less-active) forms of a hormone. Ectopic hormone secretion by a tumor can also be masked if feedback control suppresses hormone production by the normal gland of origin. Furthermore, production of more than one hormone by a tumor may present such a confusing clinical picture that the effects of each hormone are masked.

The best-known example of ectopic hormone production by a tumor is in lung cancer patients, where the tumor often secretes ectopic adrenocorticotropin (ACTH). ACTH is normally secreted by the adrenal gland, and it stimulates production of other hormones by the adrenal gland. Overproduction of ACTH results in Cushing's disease, with various clinical symptoms including high blood pressure, general weakness, water retention, glucose excretion, osteoporosis, and obesity of the trunk. When ACTH overproduction results from lung cancer, all of these clinical symptoms may be manifest, in combina-

tion with the symptoms more directly attributed to the tumor. One study found that almost half of the SCLC patients evaluated had elevated levels of ACTH in the blood, yet fewer than 2% had clinical symptoms of ACTH overproduction.⁶²

Another example of the systemic effects of ectopic hormone production can be seen in certain patients with testicular cancer. These tumors can potentially secrete ectopic chorionic gonadotropin (CG). CG is normally synthesized in the placenta of pregnant women and is present in only trace quantities in normal testis. Yet, in testicular cancer patients, CG is often detected at high levels in the blood. In fact, CG can serve as a reliable marker for metastatic disease if it is still present in the blood after surgery to remove the cancerous testes. Ectopic production of CG can result in feminization of the male patient, with symptoms including gynecomastia, the development of male breasts. High levels of CG are also associated with hyperthyroidism, since CG is structurally similar to a thyroid-stimulating hormone. Ectopic CG release is also occasionally seen in patients with lung carcinoma or hepatoma, and a syndrome of precocious puberty has been documented in young boys with liver tumors that produce ectopic CG.

Ectopic hormone production has been associated with a wide variety of tumors, including prostate cancer, hepatoma, ovarian cancer, thyroid tumors, pancreatic islet tumors, salivary gland tumors, and certain brain tumors. However, very low levels of ectopic hormone production may be associated with an even wider range of cancers. In many terminal cancer patients the end stages of the disease are associated with general organ system failure that may be the result of ectopic hormone production by the tumor.

GENERAL SYSTEMIC EFFECTS OF THE TUMOR ON THE PATIENT

One of the most common conditions associated with the late stages of cancer is a general physical wasting called cachexia. Cachexia is often attributed to loss of appetite, and it can be associated with distortion of the sensation of taste and an acquired aversion to certain foods such as meat. However, in many patients, the extent of weight loss far exceeds the deficit in quantity or quality of calories consumed. In addition to loss of body fat, there may be a striking loss of muscle mass, combined with symptoms of diabetes such as insulin resistance and abnormal glucose tolerance. In these patients, cachexia is

probably caused by something more insidious than a simple loss of appetite.

Recent research has indicated a possible biological basis for malignant cachexia.⁶³ Certain cells in the bloodstream, known as macrophages, can be stimulated to release a factor called cachectin. Cachectin selectively inhibits the activity of enzymes associated with fat storage in fat cells, and it causes fat cells to mobilize stored fats. When cachectin was added to cultured adipose cells, it rapidly and selectively turned off synthesis of fat-storage enzymes. Cachectin release was induced in mice exposed to certain bacteria, so cachectin may promote cellular defenses against invasion of the body. If the patient's body synthesizes cachectin in response to certain tumors, this could explain the wasting syndrome so common in advanced cancer.

Even in noncachexic patients, it is likely that the presence of a tumor has a strong effect on patient metabolism. Protein metabolism was characterized in nine patients with lung cancer and compared with protein metabolism in nine noncancer patients. All of the patients were matched in terms of age, weight, and smoking habits, and none of the cancer patients was cachexic. Protein metabolism of all patients was assessed by infusing a radioactive amino acid and following this tracer through various metabolic pathways. Immediately after eating, the rate of incorporation of radioactivity into protein, and the release of radioactivity by protein degradation, was higher in the cancer patients. There were no differences between the two groups in terms of energy expenditure or the pattern of nutrient utilization. Therefore, lung cancer patients show an increased rate of protein turnover, despite the fact that there was no detectable difference in energy expenditure.⁶⁴ This suggests that the tumor adversely affects patient protein metabolism by increasing the availability of amino acids for tumor growth (Fig. 27).

Several other severe systemic medical conditions can also be associated with cancer. These conditions are often lumped together under the heading of "paraneoplastic syndromes." One example is fever of unknown origin, which is probably attributable to infection. The immune system in a cancer patient may be suppressed, either by the cancer or by the cancer treatment, thus opening the door for opportunistic infection. Opportunistic infections are caused by bacteria, viruses, fungi, or other organisms, which are ordinarily either rare or benign. In certain instances, a fever cannot be explained by any identified infection, and the cause of the fever may be attributed to the cancer itself.

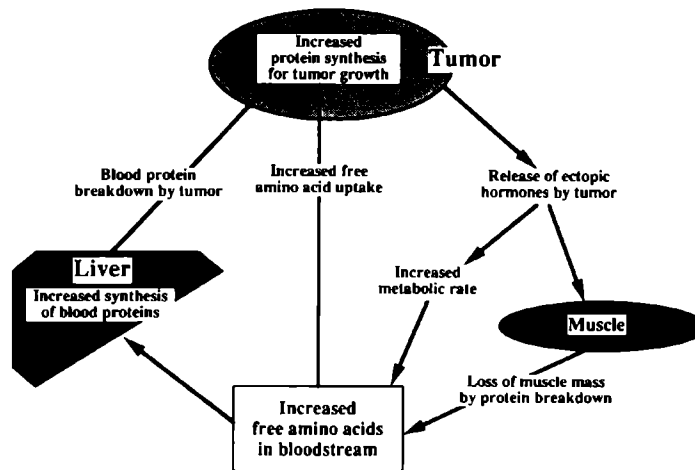


Figure 27. Summary diagram of the potential metabolic and hormonal interactions between a tumor and a patient. The release of ectopic hormones (or growth factors) by the tumor is a poorly understood aspect of this complex relationship, but it is certain that many tumors release a complex array of ectopic hormones.

Neurologic disorders can complicate the effects of cancer. Often neurologic disorders can be attributed to cancer therapy, since both radiation and chemotherapy have a strong deleterious effect on nerve cells. But certain neurologic conditions that arise are not related to therapy or to invasion or compression of nervous tissue. For example, Eaton–Lambert syndrome is characterized by weakness and fatigability, primarily of the muscles of the pelvis and thighs. This syndrome often occurs in conjunction with dry mouth, impotence, aching thighs, and loss of tendon reflexes. The syndrome is associated with cancer in 70% of all cases, with SCLC being the most frequently associated cancer. Presence of Eaton–Lambert syndrome is actually a positive prognostic factor for the patient with SCLC, because Eaton–Lambert symptoms can be manifested when the tumor itself is quite small.

Probably the most common paraneoplastic syndrome is distal polyneuropathy, characterized by limb weakness, sensory loss, and absence of tendon reflexes in the limbs. This neuropathy can be associated with degeneration of peripheral nerves, and generally does not improve after removal of the

primary tumor. Distal polyneuropathy is most commonly associated with SCLC or tumors of the breast, stomach, and thymus.

TUMOR PRODUCTS AS TUMOR MARKERS

Systemic effects caused by the release of bioactive compounds from the tumor can be a very severe problem for the cancer patient. However, under certain conditions, release of molecules by the tumor can actually aid in tumor diagnosis. If release of certain molecules by a tumor is specific to that particular type of cancer, then the presence of the molecule serves as a tumor marker.

A tumor marker is clinically useful for a variety of reasons. It may be possible to use markers to screen high-risk individuals for the presence of malignancy. A tumor marker may also provide the definitive indicator of malignancy in a patient already identified as a potential cancer patient. A marker can be used to monitor the success of therapy or to serve as an early indicator of tumor recurrence. Finally, if a tumor marker is exclusively expressed on the surface of tumor cells, then it can serve as a target for radiolabeled antibodies that might permit detection of occult metastases or direct treatment of primary tumors.

One tumor marker that is commonly seen in cancer patients is carcinoembryonic antigen (CEA). CEA was once thought to be specific for cancers of the bowel, but blood CEA levels are also elevated in cancers of the pancreas, stomach, lung, and breast. Moreover, CEA can also be elevated in certain benign conditions, including cigarette smoking, hepatitis, alcoholic cirrhosis, inflammatory bowel disease, and chronic pulmonary disease. The fact that CEA is associated with such a broad range of medical conditions tends to weaken its utility to the clinical oncologist. However, serial measurements of serum CEA can be used to monitor success of cancer therapy.

Another tumor marker that is more diagnostic of a particular tumor is prostate-specific antigen (PSA), which is associated with cancer of the prostate. PSA appears to be specific for prostatic epithelium, whether benign or malignant, since elevation of serum PSA has been reported for carcinoma of the prostate, benign prostatic hyperplasia, and prostate inflammation. Because PSA elevation is not specific for carcinoma, it may be of limited use as a screening test. But serum PSA levels may prove very useful in monitoring for metastatic disease after radical prostatectomy. Since radical prostatectomy

should remove all sources of PSA, residual PSA in the bloodstream would be strongly suggestive of metastatic disease.

CLINICAL COURSE AS A CONSEQUENCE OF TUMOR PHYSIOLOGY

Tumors produce their most obvious clinical symptoms because of local effects. Tumor cells can proliferate in an organ, destroying the normal cells of that organ and leading to a loss of normal organ function. For example, marrow replacement by leukemia cells results in reduced production of normal blood cells; invasion of cancer into artery walls can weaken these vessels and cause hemorrhage; lung cancers can compromise the gas-exchange function of involved parts of the lung; cancer of the bone causes a weakening of the bone structure, resulting in bone fractures; and hepatoma cells can replace normal liver cells and interfere with vital functions of the liver.

Alternatively, tumor cells can proliferate to such an extent that they obstruct passageways within the body. This can also lead to a loss of normal organ function. For example, colon cancer can obstruct the bowel, preventing the passage of waste material; lung tumors can obstruct the flow of air through the lung and can even block the return of venous blood to the heart; liver and pancreatic cancer can produce obstructive jaundice; abdominal tumors can swell around the ureters and cause renal failure; and brain tumors can obstruct the ventricles that drain fluid from the brain, leading to an elevation of intracranial pressure and partial loss of brain function.

Destruction and obstruction clearly can account for many cancer deaths, but they do not account for all deaths. The question then becomes, how does cancer kill? At least 50% of cancer patients die from various complications of cancer, including bacterial sepsis, opportunistic viral infections, sudden hemorrhage, or progressive failure of an organ or organ system. A relatively small number of patients die from complications of treatment, including operative mortality, or toxicity caused by chemotherapy or radiotherapy. General wasting or cachexia seems to precipitate patient death in many cases, but this is a vague term covering a variety of biologic entities and obscuring an absence of specific knowledge of cause of death.

Most tumors that ultimately prove fatal are metastatic and spread to other organs. Metastasis of a cancer is clearly a very serious situation, because metastasis increases the total tumor burden for the patient. While increased tumor burden can complicate the picture for a cancer patient, it is not, in itself,

lethal. For example, one patient recently underwent surgery to remove a benign ovarian tumor that weighed more than 100 pounds, and a 40-pound liver tumor was removed from another patient. Clearly, a large tumor burden alone is not sufficient to cause death. Conversely, a benign islet cell adenoma weighing only a gram can prove fatal if it causes untreated hypoglycemia (low blood sugar). The fact that such a small and slow-growing tumor can prove fatal may be a valuable clue to the way in which cancer affects the cancer patient.

It is fair to say that we do not know precisely how cancer kills the patient in many cases. However, an answer to this question will probably come from examining the systemic effects of cancer. Ectopic hormone production by tumors appears to be a widespread and largely unrecognized problem. Ectopic hormones can interfere with the ability of the cancer patient to maintain an equilibrium of bodily processes and may also be responsible for suppression of the immune system. Many terminal cancer patients suffer overwhelming bacterial infections or general organ system failure, as if the body were unable to self-regulate or maintain a physiological equilibrium. If physicians could learn how to maintain the physiological equilibrium of a patient artificially, perhaps by counteracting the effects of ectopic hormone production by the tumor, this could conceivably buy time to further combat cancer in the cancer patient.

CHAPTER 8

Cancer and the Immune System

The response of the immune system to disease is one of the most complex and wondrous processes in the human body. The immune system rivals the nervous system in terms of the subtle, carefully modulated, and very complicated interactions among the cells involved. Immune system dysfunction is apparent in infectious diseases such as AIDS, autoimmune diseases such as diabetes, and persistent parasitic infections such as malaria and schistosomiasis. A clear understanding of immune system function is crucial if we are ever going to conquer cancer, and eradication of other disease states also depends on a better knowledge of the immune system.

An understanding of cancer cannot be achieved without consideration of how the immune system is supposed to function to suppress tumor growth, and of how this system can become deranged in function. A patient is often able to mount an immune response to a tumor, and usually this response is capable of at least slowing tumor growth. However, the immune response is almost always unsuccessful in stopping tumor growth once a tumor forms a palpable mass. In fact, the immune response has already failed to suppress tumor growth before this point, or else the tumor would never have been able to form a mass. Thus, a diagnosis of cancer means that, to a certain extent, the immune system of the patient has already failed to kill growing tumor cells or to suppress tumor growth.

NORMAL MECHANISMS OF TUMOR IMMUNITY

Normal immune system function basically depends on four different types of cells that circulate in the bloodstream, where they form a mobile response team that can be at the scene of an emergent crisis in a very short period of time. The proper functioning of the immune system ultimately depends on the proper

functioning of each cell type, since each has a different role in the total response. The four principal cell types involved are: monocyte/macrophages, which are the first alert system of the body, without which the whole process cannot begin; T lymphocytes, which are the linchpin of the whole system, responsible for modulating the activity of all other immune cells; B lymphocytes, which manufacture antibodies; and natural killer (NK) cells, which are capable of direct attack on cells (Fig. 28).

Monocytes are large white blood cells (leukocytes) that are normally found circulating in the bloodstream or lodged in the lymph nodes, spleen, bone marrow, and certain connective tissues.⁶⁵ Macrophages are apparently a counterpart to monocytes, but they actually move through tissue in an amoeboid fashion. Both monocytes and macrophages are capable of ingesting and breaking down other cells, bacteria, bits of necrotic tissue, and foreign particles. When monocytes and macrophages encounter an object that they recognize as “nonself” (i.e., something foreign or abnormal in the body), they

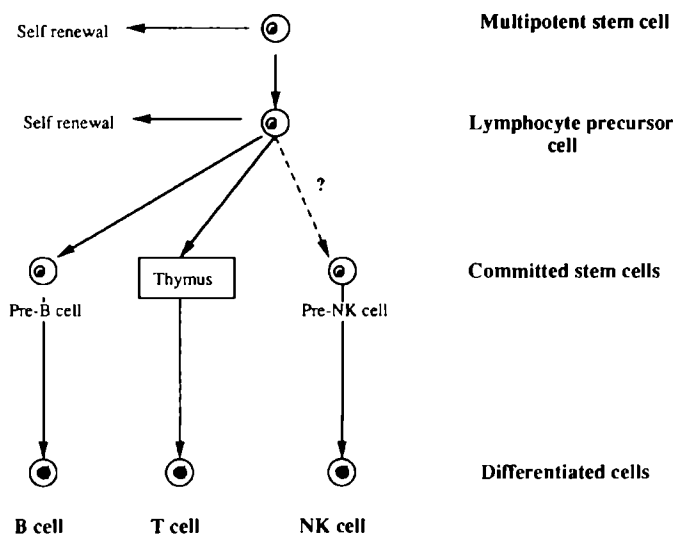


Figure 28. Diagram of a proposed genealogy of B and T cells. The multipotent stem cell is capable of producing cells that can be either hematopoietic stem cells or lymphopoietic stem cells (this diagram shows lymphopoiesis only). The lymphocyte precursor cell can give rise to B cells, T cells, or NK cells, depending on which developmental pathway the daughter cells take. The T cell must mature in the thymus, so the developmental pathway for this cell is shown leading through this organ. Once a differentiated cell is produced, it is no longer capable of generating cells of other lineages. (From J. H. Jandl, 1991, *Blood: Pathophysiology*. Reprinted by permission of Blackwell Scientific Publications.)

can engulf and destroy that object. Monocytes and macrophages are capable of distinguishing "self" from "nonself" on the basis of molecules that are typically displayed on the surface of "self" cells: if these molecules are not properly displayed, the object is assumed to be "nonself" and is attacked. Self recognition is very important because if the body's own cells are not properly recognized as self cells, they too will be attacked. If self cells are attacked and destroyed, this is an autoimmune response, which is involved in serious diseases such as diabetes, lupus, inflammatory bowel disease, multiple sclerosis, and perhaps Alzheimer's disease.

When monocyte/macrophages encounter a nonself object such as a bacterial cell, they respond to the nonself molecules, or antigens, that are presented on the surface of the bacterial cell. Interestingly, monocyte/macrophages can also attack self cells that are infected with a virus or have been transformed to a cancerous state. This attack on self cells probably arises because the self cells present unusual antigens on their cell surface. However, because these cells are still self cells, the attack on them by monocyte/macrophages may be attenuated with respect to an attack on a bacterial cell.

Monocyte/macrophages respond to nonself antigens by engulfing and processing them, then displaying the processed nonself antigens on their own cell surface. A monocyte/macrophage that is displaying processed antigen is referred to as an activated cell, and activated cells affect the immune system in several different ways. Activated monocyte/macrophages can directly interact with and stimulate specific T cells, so that these cells will also become involved in the immune response. In addition, activated macrophages secrete interleukin, a molecule that stimulates T cells, and interferon, a molecule that stimulates other macrophages. Secretion of these molecules essentially serves to amplify the signal given to the original macrophage, causing other macrophages and T cells to be recruited into the fight against the foreign intruder. An activated macrophage can also develop into a cytotoxic macrophage, which is capable of attacking a nonself or tumor cell directly. Such an attack would probably result in death for the attacked cell because the macrophage is a powerful adversary, but the mechanism of tumor cell death is unknown.

T lymphocytes are activated and recruited into the immune response only by interacting with already-activated macrophages (Fig. 29). T cells serve a variety of different roles and interact with virtually every other cell of the immune system. T cells are activated by encountering a monocyte/macrophage that is presenting nonself antigen in conjunction with the cells' own self antigens. Once a T cell has been stimulated, it undergoes a process of change

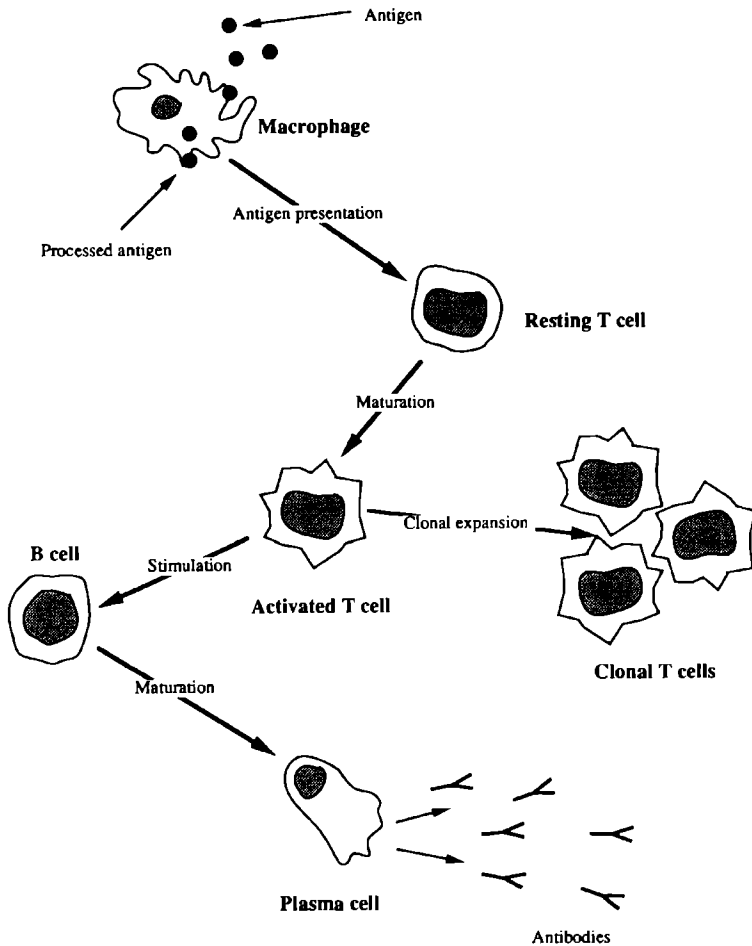


Figure 29. Simplified diagram showing the steps involved in immune response to an antigen. The macrophage is responsible for detecting antigen, processing that antigen so that resting T cells can respond to it, and presenting the antigen to an appropriate T cell. Once antigen presentation has occurred, the T cell stimulates all other processes. (From J. H. Jandl, 1991, *Blood: Pathophysiology*. Reprinted by permission of Blackwell Scientific Publications.)

that culminates in the development of several different types of T cell. About 55 to 65% of the unstimulated T cells of the bloodstream are called T4 cells; once these cells are stimulated they develop into helper T cells. Helper T cells provide helper function to other T cells, to macrophages, and to B cells, through the release of several different molecules. Helper T cells can secrete interferon, which feeds back and stimulates new macrophages to become involved in the immune response; interleukin, which stimulates another type of T cell called a

T8 cell; and several other molecules that act as attractants for macrophages. Helper T cells also stimulate B cells to begin their own particular process of maturation. The T4 cell appears to be the target for the AIDS virus, so clearly loss of this cell can cripple the immune system.

T8 cells, which are stimulated to mature by interleukin released from helper T cells, can develop into two different types of T cell. Some of the activated T8 cells develop into cytotoxic T lymphocytes (CTLs), which can directly attack tumor cells. CTL cells are particularly interesting because they can only attack cells that display tumor antigens in conjunction with self antigens. This means that CTL cells are unable to attack bacterial cells or parasites, so they may have evolved specifically to eliminate tumor cells. The remaining activated T8 cells develop into suppressor T cells, which act to suppress immune system function. Suppressor T cells can suppress the recruitment of new T cells into an immune response, they can stop CTL cells from attacking tumor cells, and they can suppress production of antibodies by B cells. Presumably suppressor T cells evolved in order to shut down the immune attack on a particular antigen after that antigen is no longer a threat. Suppressor T cells may also prevent the immune system from overresponding to a particular antigen, while failing to respond to other antigens. Suppressor T cell function can apparently become deranged in cancer patients, so that the immune response to a tumor is shut down before the tumor is eliminated. Excessive suppressor T cell activity is associated with general immunosuppression, or decrease in the immune response to all antigens, while loss of suppressor T cell activity can be associated with various autoimmune diseases.

The third cell type of the immune system, the B lymphocyte, is stimulated to mature by the helper T cells. B cells are directly stimulated by cell-to-cell interaction with helper T cells, and they are also affected by several molecules released by helper T cells. Once a B cell is activated, it multiplies and differentiates into a cell called a plasma cell. Plasma cells can secrete large quantities of antibody to whatever antigen stimulated the immune system. Antibodies produced by a plasma cell can directly mediate tumor cell killing by interacting with certain proteins in the bloodstream called complement. However, the direct attack of antibodies and complement on tumor cells appears to play a minor role in the immune response to a tumor. More important is the interaction of antibodies with other cells of the immune system, such as macrophages, T cells, and NK cells. In this type of interaction, antibody first coats the antigens displayed on the surface of a tumor cell, then these antibodies attract other cells that are able to directly attack the tumor cell. But an

overabundance of tumor antibodies can actually block the response of the immune system to a particular tumor cell, by binding to the molecules that coat macrophages and NK cells and that enable these effector cells to recognize tumor cells.

The last major component of the immune system are the NK cells. These cells are similar to CTLs, in that both cells can directly attack and lyse tumor cells. However, CTLs are restricted to attacking tumor cells that display tumor antigens in conjunction with self antigens, while NK cells are not similarly restricted. Thus, NK cells can attack a tumor cell that displays a tumor antigen, whether or not a self antigen is also displayed. The ability of NK cells to kill tumor cells does not depend on prior exposure to the tumor antigen and does not require interaction with antibodies. Thus, NK cells are indiscriminate killers of all cells recognized as nonself, and they have even been implicated in some killing of normal self cells. NK cells are stimulated to become active killer cells under the influence of interferon, which is released by T cells as well as by other activated NK cells. NK cells may play a key role in the immune response against a tumor, although NK cell activity can be overwhelmed by a rapidly growing tumor. Stimulation of NK cells may explain why an injection of interferon can sometimes stimulate an immune response against a tumor in a cancer patient. It has been proposed that NK cells play a role in maintaining a general, long-term surveillance of cells in the body, guarding particularly against virus-induced tumors. Abnormally low NK cell activity has also been associated with a tendency to develop leukemia.

When a nascent tumor is encountered by a cell of the immune system, a complex and cascading response is set off (Fig. 29). Tumor cells are probably initially recognized as foreign by monocyte/macrophages, which can infiltrate directly into a tumor mass. These cells process tumor antigens and present them on their own cell surface, thereby stimulating both T4 and T8 cells. The T4 cells rapidly develop into helper T cells, which provide helper function to other cells of the immune system. In particular, B cells are stimulated to proliferate and mature into plasma cells, which can begin to release antibodies within a relatively short period of time (hours to days). T8 cells are stimulated, and begin to attack tumor cells as soon as they mature into CTL cells. Meanwhile, NK cells are probably already attacking tumor cells, since they may form the first line of defense against tumor growth. T8 cells would not be stimulated to form suppressor T cells until the tumor cells have largely been eradicated. This rapid and massive immune response to tumor cells can result in spontaneous regression of an established tumor. However, it is unclear

whether this sort of immune response is generally successful, since evidence of success would be the absence of a tumor.

IMMUNE SURVEILLANCE THEORY

The idea that the immune system is constantly on guard against the random event of a normal cell transforming to a cancerous state is called immune surveillance theory. Although this idea is not new, it has been the subject of vigorous debate for many years. It was proposed that the immune system is ever vigilant for bacteria, viruses, and aberrant or transformed cells of the body. When these threats are encountered they are rapidly and selectively eliminated. The original theory proposed that malignant cells arise frequently in normal individuals, and that these cells are almost always eliminated by quick response of the immune system.

Evidence in favor of immune surveillance theory comes from a variety of sources. For example, certain virally induced tumors produce an immune response in animals exposed to the virus. It is possible to immunize these animals with a piece of tumor displaying the antigen, or with an inactivated virus, so that the animal becomes immune to the virus. Polyoma virus induces tumors in mice, but mice exposed to fragments of tumor implanted in the skin are able to resist the virus so that they do not develop tumors. Similarly, chickens are subject to a virally induced leukemia called Marek's disease, but immunization of chickens with a particular turkey virus can make them resistant to the chicken virus. In humans, the Epstein-Barr virus (EBV) is implicated in development of at least four different kinds of cancer: Burkitt's lymphoma, nasopharyngeal cancer, Hodgkin's lymphoma, and B-cell lymphoma. Yet EBV antibodies are found in about 90% of the population of the United States, and in virtually 100% of the population in other parts of the world, and most of these people never develop cancer. Thus, the immune system apparently succeeds in suppressing tumors induced by EBV in most persons exposed to the virus. Another observation that is consistent with immune surveillance theory is that children born with immune system deficiencies are generally more prone to developing cancer. Finally, the rare instances of spontaneous regression of a large tumor are generally thought to be evidence of immune response.

The role of the immune system in tumor suppression is further supported by the fact that immunosuppressed patients are more prone to develop cancer.

For example, organ transplant patients, whose immune system is suppressed to prevent organ rejection, have a threefold higher risk of cancer than persons who are not immunosuppressed. Transplant patients can develop a broad range of different cancers, but carcinoma of the skin, anus, or genitals, and non-Hodgkin's lymphoma are the most frequent cancers. The risk of skin cancer is elevated 4- to 21-fold relative to the general population, and the cancers that develop tend to be more aggressive. Skin cancer is usually caused by sunlight exposure, so immunosuppression may permit tumors to grow from light-damaged cells that would ordinarily be eliminated. Anogenital cancer incidence is increased more than 100-fold in immunosuppressed patients compared with the general population. Anogenital cancer is typically associated with human papilloma virus (HPV), so immune suppression may permit tumor growth from cells made malignant before immune suppression occurred. However, transplant patients might have a higher incidence of cancer for other reasons as well; perhaps chronic viruses are carried in or activated by the transplanted tissue, or there may be carcinogenic effects of the drugs used for immunosuppression.

Evidence in support of the immune surveillance theory has also come from study of patients with acquired immune deficiency syndrome (AIDS). Although the mechanism by which the AIDS virus ravages the immune system remains unknown, the T4 cell, which forms helper T cells, appears to be the target of the virus. After immunosuppression of the AIDS patient occurs, often several years into the course of AIDS, the patient becomes vulnerable to an increased incidence of cancer. Between 20 and 40% of all AIDS patients develop Kaposi's sarcoma, which is normally a very rare cancer. Kaposi's sarcoma is a highly variable tumor that develops from cells lining either the capillaries or the lymphatic system. Kaposi's sarcoma can develop in AIDS patients before there is serious compromise of the immune system, but as the immune system of the patient declines, the tumor tends to become more aggressive. AIDS patients also have an increased risk of developing non-Hodgkin's lymphoma, a malignancy of the immune system.

Although there is evidence favoring immune surveillance theory, there is also some evidence against it, and no one really knows whether or not the theory is correct. It is worth noting that both cancers that strike AIDS patients (Kaposi's sarcoma and non-Hodgkin's lymphoma) are cancers affecting cells of the circulatory or lymphatic system. If immune surveillance theory is correct, then tumors in the immunocompromised AIDS patient should arise at locations throughout the body, not just in the circulatory and lymphatic system. A

widespread incidence of cancer would be expected because all cells of the body are released from immune surveillance in AIDS patients. However, AIDS patients do not seem to suffer an increased incidence of any cancers other than Kaposi's sarcoma and non-Hodgkin's lymphoma. This suggests that immune surveillance theory may not be entirely valid.

Furthermore, if immune surveillance theory is valid, then patients diagnosed with cancer should be expected to develop a second primary tumor rather frequently. This expectation arises because the first primary malignancy indicates that immune surveillance has failed, so development of a second primary malignancy should be permitted. Long-term follow-up of cancer survivors does show that these patients have a roughly sixfold higher incidence of a second primary cancer. The second cancer often arises 4 to 6 years after treatment of the first cancer, and the second cancer is frequently either acute leukemia or non-Hodgkin's lymphoma. The fact that cancer patients have a high incidence of these cancers only, without a corresponding increase in incidence of other cancers, suggests that the second primary cancer is not the result of a failure in immune surveillance. Most cancer patients are treated with anticancer therapy that is often both immunosuppressive and carcinogenic. Thus, development of a second primary cancer may actually be a result of treatment for the first tumor, rather than a result of immune system failure.

MECHANISMS BY WHICH TUMOR CELLS ESCAPE THE IMMUNE RESPONSE

A great deal of attention has been focused on mechanisms a tumor cell may use to evade the immune system. This issue is of very broad significance, since many disease-causing organisms are able to evade the immune system, and understanding the mechanisms of immune evasion used by cancer cells might enhance understanding of immune evasion by disease-causing organisms as well.

Experiments in cell culture show that most spontaneous tumors in animals and in humans do not induce a strong immune response. This is probably because spontaneous tumors grow so slowly that there is a long period of time during which tumor cells do not encounter circulating cells of the immune system. This would give the tumor cells time to mutate to a form that is not strongly immunogenic. Analysis of the cells present in a tumor mass shows clearly that they are heterogeneous in terms of antigens displayed on the cell

surfaces. If some of these random mutations result in cells that are not strongly immunogenic, then these cells are more likely to survive an attack by immune cells. Tumor cell evasion of the immune response could thus result from a strong selective pressure placed on the growing tumor; immunogenic tumor cells are killed, leaving behind only nonimmunogenic tumor cells. The latter cells would then continue to grow, populating the tumor with a clone of nonimmunogenic progeny. This is a type of clonal evolution, with the disastrous consequence that the immune response of the body is attenuated because of selection for nonimmunogenic tumor cells.

Numerous other mechanisms of immune evasion by a growing tumor have been proposed. The immune response to a tumor may be depressed when a tumor is able to shed a large amount of antigen into the bloodstream. Thus, a large tumor that sheds massive amounts of antigen may be able to simply overwhelm the immune system and activate suppressor T cells. There is some evidence that the body may even mount an autoimmune response against those cytotoxic lymphocyte (CTL) cells that are able to attack tumor cells. This autoimmune response is mediated by suppressor T cells, which specifically recognize and kill tumoricidal CTL cells.

Tumor cells also appear to be able to release substances that modulate the immune system of the patient. The blood serum of tumor patients may contain blocking factors that interfere with the activity of various immune system cells. For example, some tumors appear to be able to release substances that are normally released by suppressor T cells, and that act to shut down the immune response to a tumor antigen. Other tumors are known to release prostaglandins, which have a strong inhibitory effect on monocyte/macrophages. The macrophages of patients with advanced tumors are often inactive against tumor cells, but this inactivity can be overcome in cell culture by treatment with certain drugs. Finally, excess antigen released by the tumor cell can block antigen binding sites on tumoricidal CTL or NK cells. Excess antigen can also potentially deplete the bloodstream of circulating antibody, by binding to antibody so that antigen–antibody complexes are excreted by the patient.

Research has suggested that a reduction in the effectiveness of NK cells may be associated with cancer progression. Decreased tumoricidal activity of NK cells could be caused by several different factors, including a decrease in binding of NK cells to tumor cells, a reduction in the number of functional NK cells, or a decrease in the rate at which NK cells kill tumor cells. Blood taken from 38 patients with lung cancer was compared with blood from 10 normal subjects for NK cell activity.⁶⁶ Patients with cancer showed a threefold reduc-

tion in NK cell activity compared with normal subjects. Although patient NK cells bound to tumor cells as well as normal NK cells, the rate of tumor cell killing by NK cells from cancer patients was reduced. When interleukin, a molecule that stimulates T8 cells, was added to patient NK cells in culture, the tumoricidal activity of these cells was significantly enhanced.⁶⁶ The mechanism that causes a reduction of NK cell activity is unknown, but diminution of NK cell activity has been seen in tumor-bearing mice and in some patients with metastatic tumors.

Recently a new mechanism was proposed for the progressive immune system depletion seen in patients with AIDS.⁶⁷ This mechanism has also been invoked to explain why certain pathogens, including both viruses and bacteria, can eventually cause a patient's immune system to fail completely, and some variation of this mechanism may be involved in inducing the immune tolerance seen in cancer patients. The AIDS virus apparently encodes a protein that acts as a "superantigen." The superantigen initially provokes a massive immune system response, but this response gradually becomes so attenuated that eventually it is totally absent. Two seemingly contradictory observations in AIDS patients have provided evidence suggesting that superantigens cause immunosuppression. AIDS patients are eventually unable to respond to antigens because of a gradual and selective deletion of all helper T cells. But deletion of helper T cells cannot be accounted for on the basis of direct T cell killing by the AIDS virus, since only 1 in 10,000 helper T cells is actually infected with virus.⁶⁷

These observations suggest a paradox, in that T cells that are not infected with the AIDS virus are nevertheless killed by the virus. This seeming paradox can be resolved if superantigens are involved in immunosuppression.⁶⁷ The surface of T cells is covered with receptors that bind selectively to antigens presented on the surface of other cells of the immune system. Since this binding is usually highly selective, only a handful of the total number of T cells are activated. However, a superantigen would be able to bind nonselectively to T cells, so that huge numbers of T cells are activated. This could result in a massive and immediate immune response to the superantigen, but stimulated T cells eventually lose their ability to respond to antigen and die. The result is total loss of the T cells that initially responded to the superantigen, and this loss takes the form of detectable "holes" in the T cell repertoire. When AIDS patients are compared with control patients who do not have AIDS, the "holes" in the T cell repertoire are clearly apparent. A superantigen protein encoded by the AIDS virus can thus suppress function even in T cells that never become

infected with virus. T cell deletion in AIDS patients is apparently a gradual process, which accounts for the slow decline in the ability of the immune system to respond to opportunistic infection.⁶⁷ Recently, it has been shown that a mouse mammary tumor virus also has superantigen properties. A portion of the viral genome encodes a superantigen that can eliminate responsive T cells and suppress the immune system in tumor-bearing mice. If such superantigens are also present in human tumor viruses, this could account for the immunosuppression seen in some cancer patients.

IMMUNE COMPETENCE IN THE CANCER PATIENT

Cancer patients often suffer partial immunosuppression associated with the late stages of their disease. This is particularly true of patients with leukemia or lymphoma, in whom there may be a significant depletion of cells associated with a normal immune response. The immunosuppression caused by cancer is often aggravated by the fact that many cancer therapies, including radiotherapy and various chemotherapies, also cause immunosuppression. Cancer patients have an increased incidence of infection with bacteria that cause pneumonia, wound infection, septicemia, septic fever, and other acute bacterial infections. Because of the weakened condition of patients with advanced cancer, and because of their impaired immune system, these bacterial infections are often extremely difficult to control. In fact, such infections are frequently the cause of death in advanced cancer patients. Infection is present in 70–80% of all leukemia or lymphoma patients in the end stages of their disease, and in 15–40% of all patients with metastatic carcinomas of any type. Patients with acute leukemia are often severely depleted of mature white cells in the bloodstream, so they are vulnerable to sudden and overwhelming infections of bacteria or fungus. One type of bacterium, *Pseudomonas aeruginosa*, is responsible for 35 to 50% of all fatal septicemias in patients with acute leukemia.⁶⁵

Immunosuppressed patients frequently become infected with “opportunistic” infections, caused by organisms that are usually not pathogenic and that are normally held in check by the immune system. For example, the fungus *Candida albicans* is a normal part of the intestinal flora of healthy individuals. However, in cancer patients this fungus can cause thrush and candidiasis, or more serious conditions including brain abscess, osteomyelitis, endocarditis, and septicemia. Thrush is an unpleasant but non-life-threatening infection of

oral tissues, while candidiasis is a general term for fungal infection at other sites including skin, mucus membranes, esophagus, vagina, or bladder. When the immune system is severely impaired, so that fungal growth is unchecked, the consequences can be devastating. Osteomyelitis, a painful inflammation of the bone marrow and adjacent bone, can be life-threatening because of the possibility of systemic spread of the fungus. Endocarditis is an acute, often fatal inflammation of the lining of the heart, and septicemia is systemic disease caused by growth of fungus in the bloodstream.

Fever is a very common manifestation of malignant disease, and it is often interpreted as part of the immune response to a tumor. Although temperature elevation can be caused by systemic effects of the cancer itself, more frequently fever is the result of systemic infection of the patient. About 70% of all cancer patients experience febrile episodes at some point during hospitalization for cancer. Patients with leukemia have fever during about 50% of their hospitalization, and patients with Hodgkin's disease have elevated temperatures 26% of the time.⁶⁸ Fever is common in patients with metastatic carcinomas of the lung, kidney, pancreas, or gastrointestinal tract, particularly when metastases develop in the liver. Specific immune responses are accelerated at elevated temperatures; for example, the rate of migration and the rate of particle engulfment by certain leukocytes are enhanced at febrile temperatures. Fever can be evoked by interleukin, the molecule released by macrophages that stimulates maturation of T8 cells into CTL cells. The mechanism of tumor-induced fever is not well understood, but there is evidence of a fever-inducing substance (probably interleukin) present in the urine of patients with Hodgkin's disease.

Recently it has been recognized that stress itself can have a suppressive effect on the immune system. A series of experiments with a cancer-prone strain of mouse has very clearly implicated stress as a significant risk factor for cancer.⁶⁹ Certain strains of mice carry several viruses that cause breast cancer in mice. If these mice are handled frequently and housed at high density with other stressed mice, the stress level of the mice rises, as shown by very high levels of cortisol in the blood. These stressed mice have an 80–100% likelihood of developing breast tumors within 8 to 18 months of birth. However, if these mice are handled infrequently and reared at low density, their stress level falls, as shown by a 94% reduction in blood cortisol. The incidence of breast tumors in mice at 400 days of age falls from 92% in the stressed mice, to only 7% in the unstressed mice. This suggests that chronic arousal, a concomitant of stress, can contribute to the development or progression of cancer in susceptible mice.

In humans, acute life stresses are associated with susceptibility to diabetes, ulcerative colitis, Graves' disease, rheumatoid arthritis, lupus, and cancer itself.⁶⁵ A prospective study of the husbands of women with advanced breast cancer found that, following the death of the wife, the husband's response to immune system challenge was reduced for two months. In another study, the relatively minor stress produced in medical students during final examinations caused a decrease in the number of circulating T4 cells and a decrease in NK cell activity. Studies have shown that, although anxiety and depression can inhibit the immune system, individuals who cope well with stress show an increase in NK cell activity. Immunosuppression is mediated by the nervous system, and the immune and nervous systems are similar in that both are designed to respond to stimuli in the environment. Certainly there can be few stresses more significant than a diagnosis of cancer, so it is anticipated that the diagnosis itself could have an effect on the patient's immune response to cancer.

IMMUNE THERAPY OF CANCER

In about one case out of 100,000, a histologically proven cancer undergoes a spontaneous regression and disappears. This phenomenon is most likely to occur in cases of neuroblastoma in children, in bladder and renal cell carcinoma, and in malignant melanoma, although there are isolated reports of spontaneous cure in other cancers as well. It is generally assumed that these spontaneous cures are cases in which the body has rejected the tumor, in much the same way as a transplant patient rejects a transplanted organ. This suggests that the patient's immune system can occasionally succeed in killing an established tumor.

Thus, there has been a great deal of research in recent years on ways to augment the patient's immune response to a tumor (Fig. 30). The presence of identifiable antigens on most tumor cells, and the existence of an active but ineffective immune response to many tumors, argues that a clearer understanding of immune system function may enable clinicians to successfully treat cancer.⁶⁵ If the mechanisms by which tumor cells evade the immune system were better understood, then it should become possible to use the body's own defenses against cancer. This could have the advantage of being a very selective therapy, so that cancer cells could be killed while sparing normal cells. This is

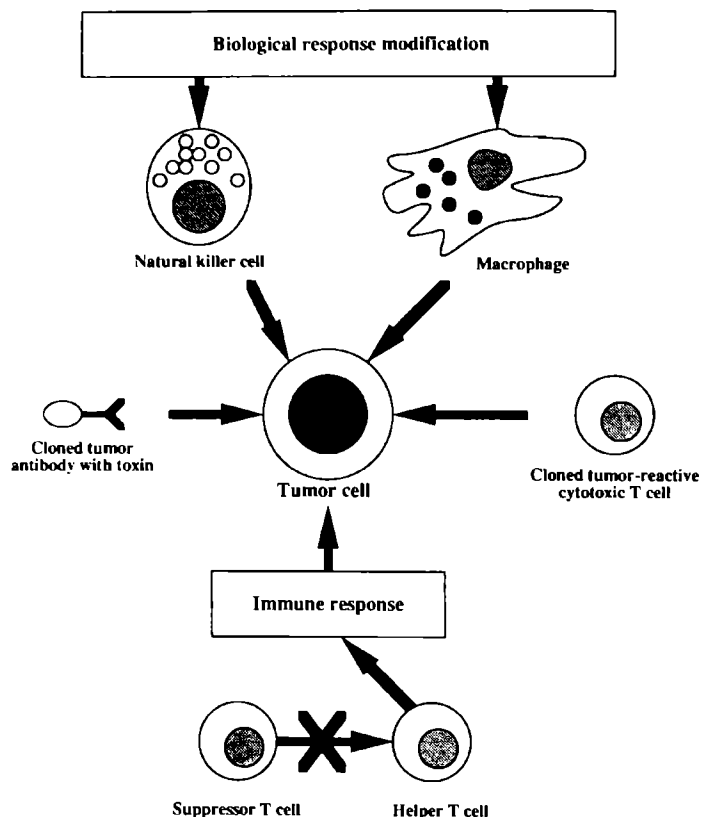


Figure 30. Possible interventions into the immune response to a tumor. Biological response modifiers could be used to activate or augment the response of natural killer cells and macrophages to the tumor cells. Monoclonal antibodies raised against tumor cells could be linked to a cell toxin and injected into the patient. Cytotoxic T cells directed against tumor antigens could also be cloned and injected. Finally, the action of suppressor T cells could be blocked artificially. (Adapted from I. Roitt, J. Brostoff, and D. Male, 1986, *Immunology*, Gower Medical Publishers.)

in sharp contrast to treatment by radiation or chemotherapy, both of which tend to kill any rapidly proliferating cell whether cancerous or not.

An early effort was made to treat cancer patients by immunizing them against the antigens expressed by their own tumor cells. This effort was unsuccessful, even with highly immunogenic tumors, probably because the cancer patient had already developed suppressor T cells that attenuated the normal immune response. However, if the suppressor T cell population could be eliminated or depleted, then this type of therapy might become effective.

Experiments in rodents suggest that if the tumor burden is reduced by surgery and if the population of suppressor T cells is reduced, then tumor immunization could be effective, especially against microscopic metastases. This is very encouraging, since micrometastases are very often a cause of tumor recurrence.

Many tumors are only weakly immunogenic, so there has also been an effort to increase the antigenicity of tumor cells. For example, human leukemia cells have been treated with a chemical to alter the cell surface, in an effort to unmask antigens already present. Other tumor cells were infected with viruses, to induce the expression of viral antigens on the cell surface and thereby stimulate an immune response. Still other experiments tried to use genetic engineering to insert genes for new antigens into tumor cells, so that these antigens would be expressed on the cell surface. There has even been an effort to augment tumor antigen expression by treating the patient with interferon, a molecule that normally stimulates macrophages to become involved in the immune response. All of these efforts have met with very limited success.

There has also been a major research effort directed toward antibody therapy of cancer. Antibodies are molecules that can cause tumor cell lysis by enhancing the interaction between tumor cells and those effector cells of the immune system that are able to kill tumor cells. Injection of tumor-specific antibodies into the bloodstream of a cancer patient might enhance the immune response of a patient to a tumor. Recent development of technology to mass-produce antibodies effective against a single antigen (i.e., monoclonal antibodies) has made it possible to administer large quantities of antibody to the cancer patient. These antibodies can be injected in their natural form, to indirectly stimulate tumor cell lysis by enhancing effector cell function. Alternatively, antibodies can be chemically linked, or conjugated, to a cytotoxic agent (e.g., cell toxin or chemotherapeutic drug) so that the antibody itself is cytotoxic. This approach does not require the participation of immune effector cells to kill the tumor cells, yet the antibody provides specificity so that tumor cells are killed while sparing normal cells. Antibody conjugates would be particularly useful if the tumor cell normally engulfs antibodies on the tumor cell surface, since this would mean that the cytotoxin has access to the inside of the tumor cell. If an antibody is conjugated to a radioactive substance, then the antibody need not actually reach each tumor cell, since radioactivity can kill at a distance. Thus, radioactive antibody conjugates might be best in a large tumor, where antibody penetration can be a problem, or in a tumor with cell-to-cell variability in antigen display.

There are a number of significant problems to solve before monoclonal

antibody therapy can be used in the clinic. Most of the antibodies used thus far in clinical trials recognize tumor-associated antigens, rather than tumor-specific antigens. This means that some cross-reactivity is expected between the antibody and certain normal cells, and the clinical utility of the antibody will be determined by normal tissue toxicity as well as by effectiveness against the tumor. Furthermore, antibodies and effector cells often do not penetrate well into a tumor mass. The reasons for this are unknown, but it is generally true that perfusion of the tumor core is poor in a large tumor mass. If blood cannot get to the tumor core, then antibodies in the bloodstream will be similarly unable to penetrate the core. Finally, tumor cells have several different ways to evade interaction with antibodies, the most obvious being simply to shed the antigen with which the antibody reacts. If tumor cells can modulate antigen expression on the cell surface, then they might be able to evade the immune response by decreasing antibody recognition of the tumor cell. There would be a strong selection for tumor cells that can shed their antigens after an antibody has recognized and bound to the antigens. Some tumor cells are apparently able to rapidly engulf antibodies that have bound to antigens on the tumor cell surface, thereby removing both the antigen and an antibody molecule. However, engulfment of an antibody might not protect a tumor cell from lethal damage if the antibody were conjugated to a cytotoxic agent.

Another approach used by clinical immunologists to augment the immune response against cancer has been called adoptive cellular immunotherapy.⁷⁰ This therapy is based on the tumor-killing ability of T cells that are injected into the patient and “adopted” as a part of the patient’s immune system. The source of these T cells must be the patient (or an identical twin), because otherwise the immune system would respond to the injected T cells as “nonself,” and would kill the injected cells before they could have an effect on the tumor cells. An approach used with solid tumors has been to excise the tumor and harvest tumor-specific T cells that have infiltrated into the tumor mass. These tumor-specific T cells are then brought into cell culture, stimulated with interleukin, and the cell population is expanded into various T cell clones. The T cell clones are then injected back into the patient, often after the patient has been treated with chemotherapy to reduce the population of suppressor T cells. Interleukin, which normally stimulates production of CTLs, has been given to patients in huge amounts to enhance survival and stimulate growth of infused T cells. Early results from clinical trials of adoptive immunotherapy have shown that T cells are able to localize to tumor sites, infiltrate into the tumor mass, and even cause a significant therapeutic effect.

A great deal more research is required before adoptive immunotherapy can be routinely used in the clinic, but the initial findings have stimulated a flurry of research. A major problem with this type of therapy is persistence in the patient of suppressor T cells that reduce the effectiveness of infused T cells. Presently, nonselective ways are used to decrease the population of suppressor T cells, but a way to specifically suppress the suppressor T cells is needed. Another problem is that T cells are harvested from the tumor and amplified in an indiscriminant manner. If a technique could be developed to select tumor-reactive T cells from the tumor, instead of merely tumor-specific T cells, this might substantially improve clinical efficacy. At present, amplified T cells could include helper T cells or even suppressor T cells, as well as CTLs, so selective amplification of CTLs before infusion into the patient might increase tumor kill.

Treatment of freshly harvested tumor-specific T cells with very high doses of interleukin has led to the production of a new type of cell, called a lymphokine-activated killer (LAK) cell.⁷⁰ LAK cells are similar to NK cells, but they are generated only when T cells are exposed to interleukin levels in cell culture that are far higher than are naturally present in the body. LAK cells apparently do not react to specific tumor antigens, but they are able to lyse transformed cells specifically by an unknown mechanism. Administration of LAK cells together with interleukin can cause significant tumor regression in some patients, but the LAK cells are not absolutely specific to the tumor, and there may be general toxicity to the patient. Several ways to increase the specificity of LAK cells for tumor cells are being explored, and LAK therapy is generally quite promising.

Typically, adoptive immunotherapy requires a long time to produce tumor regression, so the infused cells must be long-lived to have effect. If mature tumor-reactive T cells are infused into a patient, the immune response is expected to last only as long as the T cells survive. A recent advance in experimental immunology has been the ability to reconstitute the whole immune system in severely immunodeficient mice by infusion of "stem cells" that are capable of dividing to produce all of the cells of the immune system.⁷¹ Since stem cells are essentially immortal, infusion of these cells might have a long-lasting effect on the immune response in a cancer patient, provided that immune suppression is prevented. If reconstitution of an impaired immune system becomes possible in the clinic, this might enable physicians to directly stimulate an immune response to a tumor, or to repair damage to the immune system resulting from tumor therapy.

CHAPTER 9

Physiological Basis of Cancer Therapy

Current techniques of cancer therapy are generally based on an understanding of principles of cancer biology, although in certain cases known principles of cancer do not support or define current techniques of therapy. Scientists engaged in cancer research are generally interested in gaining an increased understanding of the basic biology of cancer, to better determine how tumor biology can be exploited to eradicate the disease. But many areas of ignorance remain, many cancers cannot be treated with an expectation of cure, and sometimes the advances in cancer therapy seem merely incremental.

SURGERY

Surgery is the oldest therapy for cancer and it is still used today as primary therapy for more than 75% of the cancers deemed to be curable. Surgery to excise the tumor mass will probably remain in the front line of defense against an established tumor, because it is a rapid and effective way to reduce tumor bulk. Even if chemotherapy or immune therapy of cancer achieves astonishing new successes in the future, it is likely that surgical reduction of the tumor mass will remain a major part of an effort at tumor cure. Otherwise, the sheer number of cells in a tumor mass could overwhelm the ability of even a highly effective therapy to kill all tumor cells.

A tumor mass 1 mm in diameter is comprised of approximately 10^6 (1,000,000) cells. This mass is still too small to be detected by even the most sensitive medical imaging techniques available, unless the existence of the tumor is already known from clinical effects. By the time most tumors are diagnosed, they have reached a mass of 10^8 (100,000,000) cells or more. This is an enormous number of cells to be eradicated by any therapeutic approach used

alone. But if surgery is used to debulk the tumor, then the number of remaining tumor cells may be low enough that follow-up therapy could eradicate them.

For example, if we assume that a glioma in the brain is diagnosed when it achieves a diameter of 2 cm, then the tumor mass probably contains roughly 10^9 cells. Because metastasis is very rare in these tumors, surgery could potentially be curative if all of the tumor cells in the primary tumor could be excised. But surgical cure is highly unlikely for glioma, because gliomas are very invasive. Tendrils of glioma cells would be left behind by the surgeon, in an effort to avoid causing significant deficits to the mental capacity of the patient. If surgery were able to remove as much as 99.999% of the tumor cells, then 10^4 (10,000) tumor cells would be left behind. Since a single surviving tumor cell may be enough to regenerate a tumor, surgery would be ineffective at obtaining a cure in this case without adjuvant therapy. Currently, adjuvant chemotherapy is always used to treat glioma patients after surgery, but it is usually not effective at killing all remaining tumor cells. However, at some point in the future, it may be possible for adjuvant chemotherapy or immune therapy to obtain a cure, provided that the tumor burden is reduced to about 10^4 or 10^5 tumor cells. Since surgery is the quickest way to achieve this great of a reduction in tumor cell burden, it is likely that it will remain a major weapon in the war against cancer.

Surgery is currently used to fill a variety of different therapeutic roles. In the most favorable cases surgery can be curative, but this is rare. Surgery is also used as a way to stage cancer, or to make a definitive diagnosis of cancer by obtaining a biopsy sample for histological examination. Occasionally, in cases of premalignant lesions, surgery is used as a preventive measure for cancer. Probably the most widely accepted example of this is prophylactic excision of the colon and rectum in patients with familial adenomatous polyposis, a hereditary disease that causes a greatly elevated incidence of colorectal carcinoma. Surgery is often used as treatment for local recurrence of a tumor, especially when there is no evidence of distant metastasis. Surgery may also be used in some cases merely to palliate symptoms associated with cancer. The most widely accepted type of palliative surgery is surgery to decompress the spine in cases where a tumor is causing spinal cord compression, or surgery to remove a tumor causing bowel obstruction. Only very rarely is surgery currently used primarily to reduce tumor burden, so that second-line therapies can be used. This is because the adjuvant therapies now available are not yet reliable enough to be trusted to kill all of the tumor cells remaining after surgery.

Curative surgery usually requires excision of a wide margin of normal-appearing tissue around the tumor, in order to be sure that invasive tumor cells are removed. Often, curative surgery will require excision of involved regional lymph nodes in addition to the primary tumor itself. If there is any chance that regional lymph nodes are involved, they will almost certainly be removed since there is little associated mortality from this procedure. Extensive study of the recurrence patterns of different tumor types has given surgeons a fairly clear understanding of the normal margins required for cure of a particular tumor type. In some cases, excised pieces of tumor may be reviewed by a histologist during surgery, to determine the appropriate margins around a tumor. In general, wide margins around a tumor are preferred, provided that loss of normal tissue does not cause an increase in patient mortality. But curative surgery is limited in that many cancers are diagnosed at a time when complete surgical excision is no longer possible. If the tumor has invaded vital structures or organs, then excision of these structures may be impossible. Furthermore, surgery is only effective for treatment of localized or nonmetastatic disease.

Diagnostic surgery can include biopsy surgery, exploratory surgery, laparoscopy, and surgical staging. Biopsy surgery is done with the goal of obtaining a sample of suspect tissue, for examination by a histologist trained to determine whether that tissue is cancerous or not. The major considerations during biopsy surgery are that an adequate sample of tumor tissue be obtained, that biopsy not make curative surgery any more difficult, and that biopsy not increase the likelihood of tumor metastasis. Exploratory surgery is used less frequently now than in the past, because medical imaging techniques have improved so markedly. Often laparoscopy is now used where exploratory surgery would have been used in the past. Laparoscopy is a type of exploratory surgery in which an optical instrument is inserted into the abdominal cavity through a small incision. This instrument can be used to obtain a biopsy sample or to directly examine the site of a suspected tumor. Surgical staging can be used to determine the appropriate therapy for certain types of cancer whose progression is difficult to assess without direct inspection, while "second look" laparotomy may be used in certain cases where local recurrence is deemed highly likely.

Recently, laser surgery has been used with increasing frequency in certain cancers. Laser surgery is very good at cutting into tissue with great precision, minimizing bleeding from small vessels during surgery, and actually vaporizing small tumors. The precision of laser surgery minimizes tissue trauma and can allow more rapid healing and shorter hospital stays. Furthermore, laser

surgery can be combined with chemotherapy because some agents are activated by light.

CHEMOTHERAPY

Cancer chemotherapy began in the 1940s with the introduction of nitrogen mustard, and there are now over 40 different chemotherapeutic agents available for treatment of various cancers. Chemotherapy has had its greatest successes against cancers of the bloodstream (leukemias), probably because cells in the blood are literally bathed in drug when an intravascular chemotherapeutic agent is injected. Furthermore, since most of the early experimental models used for the study of chemotherapeutic efficacy were animal models of leukemia, there is a greater understanding of the problems associated with using chemotherapy to treat leukemia (Fig. 31).

The various chemotherapeutic agents fall into several broad categories⁷²:

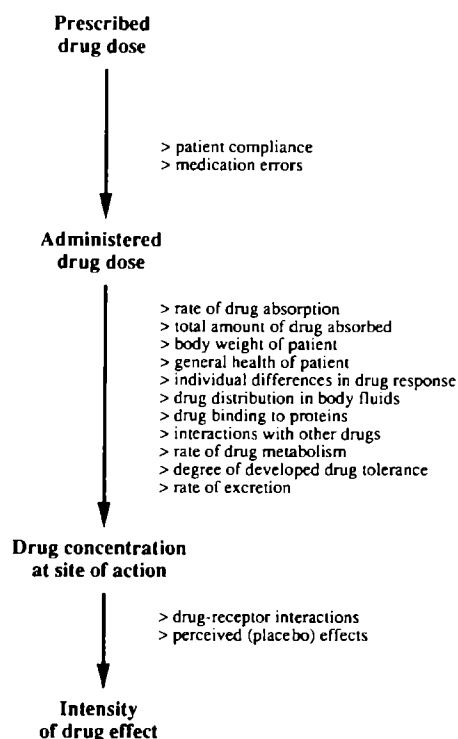


Figure 31. Factors that affect response of the individual patient to drug therapy. These factors determine the relationship between a prescribed drug dose and the intensity of drug effect. (From K. L. Melmon, 1980, *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, edited by K. L. Gilman *et al.* Reprinted by permission of McGraw-Hill, Inc.)

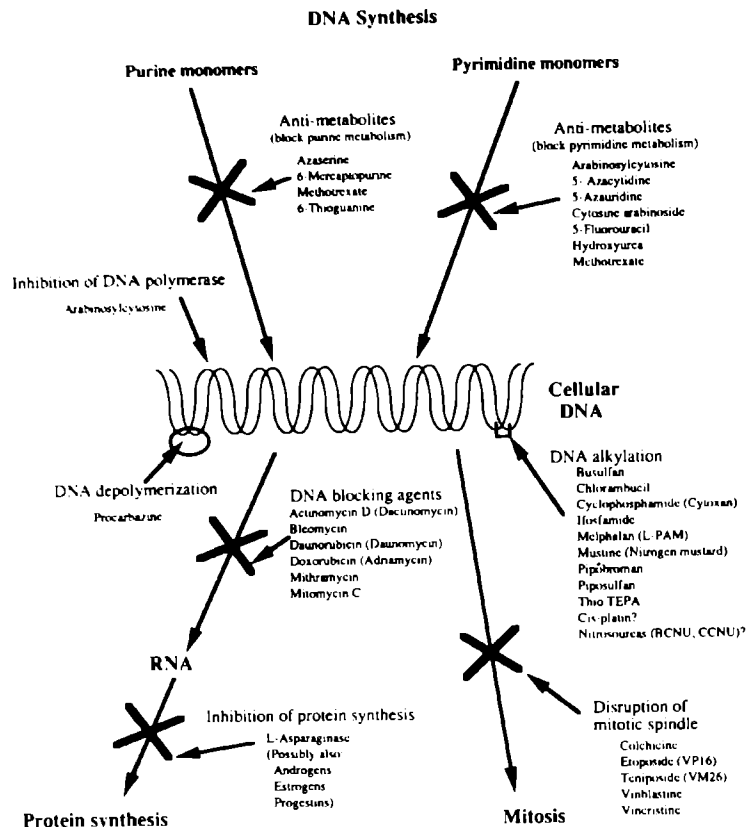


Figure 32. Proposed mechanisms of action of various chemotherapeutic agents at the cellular level. All of the various agents shown interfere one way or another with the ability of a cell to replicate DNA, undergo mitosis, or synthesize the enzymes required for cellular metabolism. In most cases, the precise mechanism by which a particular drug works is unknown, but there is a general understanding of what processes are interfered with by the drug.

1. Alkylating agents (Fig. 32). These agents directly damage DNA in the cell nucleus, often by causing the two strands of the DNA molecule to cross-link to each other. Most cells have the capacity to excise and repair damaged DNA, but certain types of damage are irreversible and many tumor cells have a reduced capacity to repair DNA. Examples of alkylating agents include: busulfan, chlorambucil, cyclophosphamide (cytoxan), ifosfamide, mustine (nitrogen mustard, mechlorethamine), melphalan (L-PAM), pipobroman, pipsulfan, and thioTEPA.
2. Antimetabolites. These agents are chemical analogues of certain

compounds required for DNA synthesis. Antimetabolites are capable of replacing a required compound and blocking the metabolic pathway in which they become involved. For example, purines and pyrimidines are required for synthesis of DNA, and most antimetabolites inhibit the synthesis of one or the other of these building blocks. Examples of antimetabolites include: 5-azacytidine, azaserine, 5-azauridine, cytosine arabinoside, 5-fluorouracil, 6-mercaptopurine, methotrexate, and 6-thioguanine.

3. Inhibitors of cellular mitosis. These agents interfere with the polymerization of microtubules, so that dividing cells cannot separate their chromosomes into daughter cells during cell division. Examples of mitosis inhibitors include: colchicine, etoposide (VP16), vinblastine, and vincristine.
4. Antitumor antibiotics. These agents bind to DNA and block the synthesis of RNA from the DNA molecule. This prevents the synthesis of new proteins, and prevents the cell from regenerating proteins destroyed or inactivated in the course of normal cell metabolism. Examples of antitumor antibiotics include: actinomycin D (dactinomycin), bleomycin, daunorubicin (daunomycin), doxorubicin (Adriamycin), mithramycin, and mitomycin C.
5. Miscellaneous agents. These agents are cytotoxic by a wide range of mechanisms, and in some cases the critical target of the drug is unknown. Examples of agents classified as miscellaneous include: carmustine (BCNU), cisplatin (CDDP), dacarbazine, hexamethylmelamine, hydroxyurea, L-asparaginase, lomustine (CCNU), procarbazine, and streptozotocin.

Most chemotherapeutic agents are able to kill a relatively constant proportion of cells in a tumor. Typically, chemotherapy kills between one and three long orders (10%–99.9%) of tumor cells, but it is almost never possible to kill all cells of a tumor. Thus, a tumor mass of 10^8 cells might be reduced to 10^5 – 10^7 cells, but it will not be cured, while a tumor mass of 10^3 cells might be very nearly cured by chemotherapy. If repeated cycles of a chemotherapeutic agent are given, the total cell kill achieved will usually be greater than possible with a single cycle. Thus, a tumor mass of 10^8 cells given one cycle of a chemotherapeutic agent might have 10^6 residual tumor cells, but after two cycles there might be only 10^4 residual cells. Repeated cycles of a chemotherapeutic agent seldom achieve a truly additive effect, because chemotherapeutic agents tend to

spare cells that are inherently resistant to the drug. In order to obtain a cure by chemotherapy, the tumor must be treated with a maximum tolerable dose of the drug and must ideally be treated when the tumor mass is quite small.

Chemotherapy can kill tumor cells without killing the patient because cells are not all equally sensitive to the drugs. At clinically relevant doses, most chemotherapeutic agents are toxic only to cells that are proceeding through the cell cycle, and drugs are often toxic only to cells at a specific point in the cell cycle. Since the proportion of cells that are actively cycling tends to be higher in tumors than in normal tissues, this means that tumors are inherently more vulnerable than most normal tissues to the effects of chemotherapy. However, the cells of certain normal tissues also proliferate very rapidly, and these normal cells may be strongly affected by chemotherapy. This accounts for why chemotherapy is often associated with nausea and vomiting (toxicity to cells of the intestinal tract), changes in the proportions of components in the blood (toxicity to cells of the bone marrow), and loss of hair (toxicity to hair follicle cells). However, the rate of cell division in a tissue is not the only factor affecting the toxicity of chemotherapy. While cells of the intestinal lining, bone marrow, and scalp can be strongly affected by chemotherapy, they are usually not killed to the same extent as tumor cells. This is because tumor cells and normal cells differ in the rate and extent to which they can repair cell damage caused by chemotherapy.

Chemotherapeutic agents can have many different effects besides killing tumor cells. Drug toxicity to normal tissues has been separated into several different categories: immediate, early, delayed, and late.⁷³ Immediate toxicity includes those effects such as nausea, vomiting, inflammation of a vein (phlebitis), increased concentration of uric acid in the blood (hyperuricemia), or kidney failure, all of which can occur within hours of treatment. Early toxicity is that toxicity that occurs within days to weeks of treatment, including reduction in white blood cells or blood platelets, hair loss, or diarrhea. Delayed toxicity can occur weeks or months after treatment, when such effects as anemia, aspermia (absence of semen), liver damage, or fibrosis of the lung may be observed. Finally, late toxicity occurs months to years after therapy, and can include sterility, atrophy of the testicles or ovaries, or induction of a second primary (iatrogenic) cancer by the chemotherapy.

In many cases, selective toxicity of a chemotherapeutic agent to a particular tissue or organ limits the maximum tolerable dosage of that drug. For example, doxorubicin (Adriamycin) is selectively toxic to the heart, and patients given high doses of doxorubicin are vulnerable to a form of cardio-

myopathy that can occur years after administration of the drug. Cyclophosphamide can cause cystitis, an inflammation of the bladder, and a sharply increased risk of bladder cancer in patients, presumably by selective toxicity to cells of the bladder. Melphalan is selectively toxic to cells of the bone marrow, so that patients given high-dose melphalan may need to be “rescued” from the effects of the drug by receiving a bone marrow transplant following drug therapy. Many chemotherapeutic agents (e.g., cyclophosphamide, cytosine arabinoside, methotrexate) are powerful suppressants of the immune system, so vulnerability to infection may lessen the survival rate of patients given high-dose chemotherapy. It is often the selective toxicity to an organ that determines the maximum dosage of a chemotherapeutic agent given to a patient.

Determination of the rate at which drugs are delivered through the bloodstream to a tumor, and of the maximum concentration of drug achieved in the tumor, is critical to improving the success rate of chemotherapy.⁷⁴ Yet clinical pharmacokinetics, the study of drug movements within the patient, is a field of study still in its infancy (Fig. 33). Despite recent efforts to characterize drug movements in patients, no one really knows the extent to which pharmacokinetics limits the cure rate achievable by chemotherapy.

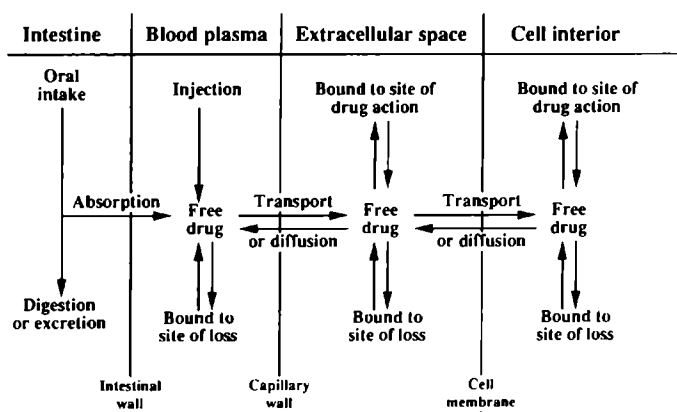


Figure 33. Schematic diagram of the route a drug has to follow in gaining access to a cell. The drug can be taken orally and absorbed through the intestine (or stomach) wall, or it can be injected into the bloodstream. In any case, the drug has to move from the plasma through the extracellular space to the cell interior. Along the way the drug can be lost through inappropriate binding to a site of loss, or the drug can bind to a site of drug action. The site of drug action can be a specialized receptor or a particular cell component. Only if the drug binds to the site of action in sufficient quantity will it have the desired effect.

Delivery of drugs to the brain provides the clearest example of how an understanding of pharmacokinetics could aid in the treatment of cancer. Brain tissue is separated from the bloodstream by a structure called the blood–brain barrier, which is formed by the endothelial cells lining brain capillaries. Molecules that are at high concentration in the bloodstream may never reach brain tissue at all if they are unable to cross the blood–brain barrier. Since this barrier can physically exclude certain molecules (e.g., water-soluble chemicals), a tumor growing in the brain may be provided with a sanctuary, where it is safe from the effect of water-soluble drugs. However, the blood–brain barrier is unable to exclude lipid-soluble drugs, so these drugs may have good access to brain tissue. This accounts for why the most widely used chemotherapy for brain tumors is BCNU, a lipid-soluble drug.

During the years since the inception of chemotherapy, there have been several notable trends in drug use.⁷⁵ When chemotherapy was first introduced, it was used only for advanced or recurrent cancers that had failed all other therapies. But there has been a trend toward using chemotherapy sooner in the course of patient therapy. There has also been a trend toward routine use of combination chemotherapy, with two or more agents given to the patient at the same time. The rationale for this is that, while tumor cells may be resistant to one of the agents used, they are unlikely to be resistant to all of them, especially when the effects of the different agents are combined. Another trend has been toward using ever higher doses of chemotherapy, while skillfully controlling treatment side effects. Finally, there has been a trend toward the use of chemotherapy as an adjuvant, or follow-up, to “curative” primary therapy. The motivation for adjuvant chemotherapy is that the drug may be able to kill metastatic tumor cells before they can form a clinically significant tumor, even if the existence of metastatic cells is undiagnosed. Adjuvant chemotherapy is used most frequently in cases where the primary tumor is likely to have seeded occult metastases by the time diagnosis is made. But, in theory, adjuvant chemotherapy should also have a higher probability of success after surgical debulking of a tumor that cannot be completely excised. This is because the proportion of growing cells in the residual tumor is high, because drug penetration of the residual tumor should be good, and because there is less opportunity for drug resistance to develop in the residual cells.

Experience over the last decade has shown that combination chemotherapy is generally more effective than single-agent chemotherapy. Combination chemotherapy usually involves a mixture of three or four of the most active agents, given together to obtain synergy. Typically, these agents are given at

very high dose levels with an expected toxicity to blood cells. For example, in patients with small-cell lung carcinoma (SCLC), chemotherapy is given for an induction period of 6 to 8 weeks, then the patient is restaged to determine if the tumor has responded to therapy. Response can be categorized as "complete clinical remission," with disappearance of all clinical signs of tumor, "partial remission," with a regression of tumor size and an improvement in clinical signs, and "no response" or tumor progression. After restaging, maintenance chemotherapy is given for periods of 6 to 12 months to SCLC patients showing complete or partial remission. If patients are still in complete remission after 6 to 12 months, chemotherapy is stopped. Patients with a partial remission are generally kept on the same regimen until the tumor begins to progress again, at which time the patient may be switched to another regimen. Patients not responding to therapy, or those with a progressing tumor, are switched immediately to a new chemotherapeutic regimen.

High dose levels are crucial for obtaining the best results from combination chemotherapy. However, the toxicity associated with high-dose combination chemotherapy can be overwhelming. In SCLC patients, physiologic staging is done to determine whether patients can tolerate intensive combination chemotherapy. Intensive chemotherapy is reserved for ambulatory patients, with no prior chemotherapy or radiotherapy, adequate cardiovascular, liver, and kidney function, and no other major medical problems. Even in this highly select group of patients, the overall mortality rate from intensive combination chemotherapy is about 5%, even at major medical centers.

Chemotherapy is currently the only therapy that offers the possibility of treating occult metastases of cancer as well as treating the primary tumor. For example, adjuvant chemotherapy is usually given to the breast cancer patient after surgery, to cure or control distant metastases. There is now a trend toward very aggressive adjuvant chemotherapy following breast cancer surgery, because 5-year survival in the absence of such treatment is not good. The National Institutes of Health reached a consensus in 1985, stating that chemotherapy should become standard for premenopausal breast cancer patients whenever there is lymph node involvement. Several clinical studies suggest that adjuvant combination chemotherapy can significantly improve relapse-free survival in node-positive breast cancer patients. Combination chemotherapy can also improve local control to the same extent as in patients who receive postoperative radiation therapy. From 1976 to 1981 there was a 20% decline in breast cancer mortality in women less than 50 years of age. This decline occurred at a time when breast cancer mortality had actually been predicted to increase. This

divergence between predicted and realized breast cancer mortality has been attributed to the success of combination chemotherapy in premenopausal women.

The efficacy of chemotherapy is now well established in certain cancers. Cancers that can potentially be cured by chemotherapy include: acute lymphoblastic leukemia, Burkitt's lymphoma and several other high-grade lymphomas, Hodgkin's lymphoma, choriocarcinoma, Wilms' tumor, and testicular tumors. Chemotherapy may provide a good response rate (but not a good cure rate) in: adult acute leukemia, breast cancer, SCLC, chronic leukemia, various lymphomas, ovarian cancer, and soft tissue sarcoma. Chemotherapy is presently relatively unsuccessful at producing remission in: cervical cancer, non-SCLC lung cancer, gastrointestinal or colorectal cancer, head and neck cancer, and melanoma.⁷²

TUMOR RESISTANCE TO CHEMOTHERAPY

The resistance of tumors to chemotherapy is currently one of the most important and difficult problems in clinical oncology. Failure of chemotherapy can result from one or several interacting factors (Fig. 34): chemotherapeutic agents may not reach cells in toxic concentrations because of insufficient delivery through the bloodstream; tumor cells may not cycle rapidly or they may rest in a resistant part of the cell cycle; intracellular concentrations of drug may be low because of reduced drug uptake, increased drug efflux, or rapid intracellular binding; presence of an intact blood-brain barrier may provide the tumor with a sanctuary; or tumor cells may be biochemically resistant because of rapid degradation of the drug, failure to activate the drug, resistance of the target molecule to drug action, or repair of drug-induced damage.⁷⁶

A great deal of study has been devoted to the mechanisms by which tumor cells resist the effects of chemotherapy. In some cases, most notably colon cancer, the tumor cells seem to be inherently resistant to drug therapy before they are ever exposed to drugs. In other cases, particularly SCLC, the tumor may initially respond well to drug therapy, but the tumor generally recurs in a form resistant to those drugs that were once effective. Development of drug resistance following treatment probably results from the fact that the drug kills vulnerable cells, leaving behind those cells that were inherently resistant to treatment. This is an example of selection resulting in clonal evolution of the tumor.

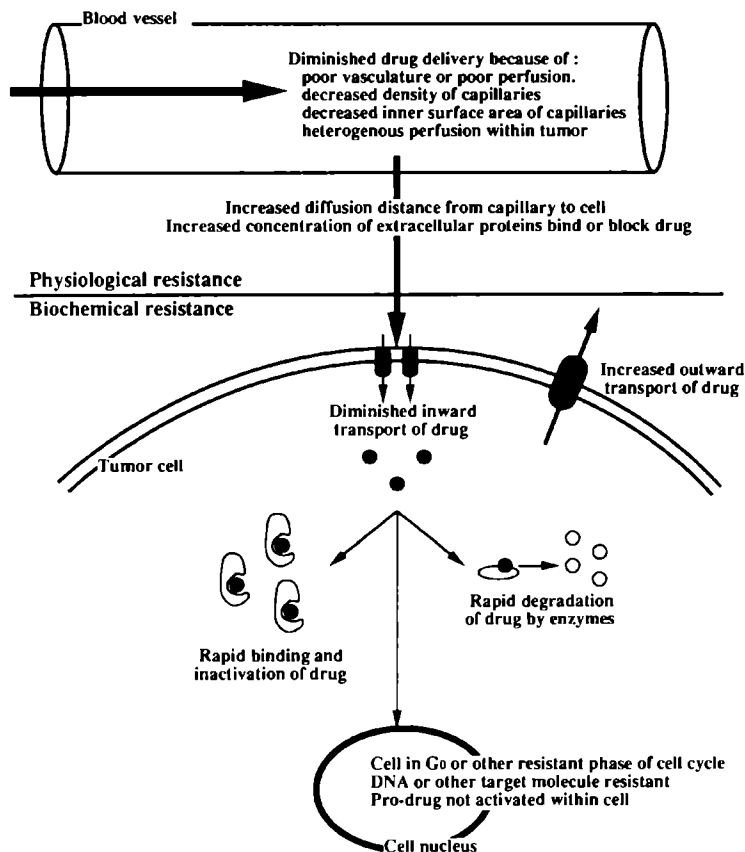


Figure 34. Summary diagram of mechanisms of tumor resistance to chemotherapy. Resistance to chemotherapy can be either physiological or biochemical in nature: physiological resistance is resistance based on the unusual physiology of a tumor, and can include any of the mechanisms above the horizontal line, whereas biochemical resistance is resistance based on the unusual biochemistry of a cancerous cell and can include any of the mechanisms below the horizontal line.

Under certain circumstances, tumor cells may show resistance to several different chemotherapeutic drugs simultaneously, a phenomenon that is called multidrug resistance. Multidrug resistance is seen in cases when a patient is treated with a drug such as doxorubicin, which succeeds in causing an initial regression or remission of the tumor. Subsequently, the tumor recurs in a form that is resistant to a range of different chemotherapeutic agents, not just the doxorubicin that had been successful at first. After recurrence, the tumor may be resistant to actinomycin D, vincristine, and vinblastine, as well as to further massive doses of doxorubicin. These different drugs share no chemical similarity beyond the fact that all are poorly soluble in water, and they all use

different mechanisms to kill tumor cells. The fact that exposure to one drug can make tumor cells resistant to many drugs is a frightening problem whose basic mechanism is still not well understood.

When multidrug-resistant cells are exposed to a particular drug, that drug appears to enter resistant cells as easily as it enters sensitive cells. But if resistant cells are then washed and placed in medium free of drug, the drug moves back out of the resistant cells more rapidly than it moves out of the sensitive cells. If the ability of resistant cells to transport drug out of the cells is somehow blocked, then resistant cells become drug-sensitive. These observations imply that resistance is conferred by a transport protein that acts to clear drug out of the cell. Careful study of the cell membrane of drug-resistant cell lines has shown that these cells have an unusual membrane protein called permeability- or P-glycoprotein. In cultured human lung tumor cells, appearance of multidrug resistance is correlated with decreased drug accumulation and increased expression of P-glycoprotein.⁷⁷ This protein is present in low quantity in normal, drug-sensitive cell lines, where it serves an unknown function. But P-glycoprotein can be present in high quantity in cells that are highly resistant to drugs, and P-glycoprotein abundance generally correlates with drug resistance.

In many highly resistant cell lines, the multidrug resistance gene (*MDR1*), which codes for P-glycoprotein, is amplified. Cells with an amplified *MDR1* gene have an increased number of gene copies that are used to synthesize P-glycoprotein. There is now compelling evidence that the product of the *MDR1* gene is directly responsible for cellular resistance to chemotherapy. When DNA containing *MDR1* was artificially introduced (transfected) into a human melanoma cell line, the cell line showed a striking increase in resistance to a wide range of drugs. The product of the *MDR1* gene enhanced resistance of transfected melanoma cells to gramicidin D by 300-fold, to vincristine and actinomycin D about 100-fold, to doxorubicin and VP16 by 10-fold, and to colchicine by 6-fold.⁷⁸ Furthermore, transfection of normal bone marrow cells with the *MDR1* gene makes these cells highly resistant to taxol, an experimental drug that is used in treatment of ovarian cancer.⁷⁹ These latter results are important because bone marrow cells are normally very sensitive to chemotherapy, and toxicity to bone marrow often limits the maximum drug dose given to the patient. If bone marrow cells were removed and deliberately transfected with the *MDR1* gene before chemotherapy, the patient might be able to tolerate higher doses of chemotherapy, and might thereby have a higher likelihood of achieving tumor cure.

There appear to be other mechanisms by which tumor cells can resist the effects of chemotherapy. Cells normally contain a chemical called glutathione, which serves a general protective function in the cell. Glutathione is capable of binding to certain highly reactive chemicals called free radicals, which may be generated in low abundance by normal processes within the cell. However, both radiation therapy and certain forms of chemotherapy are cytotoxic because they generate massive quantities of free radicals within the cell, and these free radicals bind to and destroy cellular DNA. Tumor cells with an increased concentration of glutathione in the cell cytoplasm are able to detoxify free radicals before the radicals have a chance to damage DNA. Thus, tumor cells with an increased concentration of glutathione are more resistant than normal cells to the effects of both radiotherapy and chemotherapy, since both of these treatments kill cells by generating free radicals. Treatments that deplete the cellular content of glutathione tend to make cells more sensitive to the effects of chemotherapy. Tumor cell glutathione concentration can actually be lowered by treatment of cultured cells with a relatively nontoxic chemical called buthionine sulfoxamine (BSO). Whether or not treatment with BSO can potentiate the effects of chemotherapy in patients is unknown, but BSO does potentiate the effect of melphalan in treating ovarian cancer in mice.

A better understanding of the mechanisms by which tumor cells resist chemotherapy may help the clinical oncologist to prevent the development of drug resistance by a tumor. Tumor cells are more likely to acquire drug resistance if they are exposed to low dose levels of drug for an extended period of time. This is consistent with the fact that bacteria become resistant to penicillin if they are exposed to chronic, subtoxic levels of antibiotic. The implication of this congruency for the clinical oncologist is that tumors should be treated with short-term intensive doses of chemotherapy, rather than long-term low dose levels of drug. Our understanding of tumor drug resistance further suggests that combination chemotherapy is less likely to result in drug resistance than single-agent chemotherapy.

POTENTIAL TARGETS FOR CHEMOTHERAPY

Most anticancer drugs used in the clinic today are selectively toxic to tumor cells merely because tumor cells proliferate more rapidly than most normal cells. However, a thorough understanding of cancer biology suggests that many tumor cells share certain properties that could make them vulnerable

to therapy. For example, a tumor cannot grow past a certain critical size if it is unable to induce growth of new blood vessels into the tumor mass. Angiogenesis requires that the cell division rate of endothelial cells be stimulated 20- to 2000-fold, with respect to endothelial cell growth rate in normal tissues. The fact that new blood vessel growth is required of all tumors able to form a mass larger than about 1 mg (10^6 cells) means that tumor growth could potentially be blocked if angiogenesis were somehow inhibited. Thus, inhibition of tumor angiogenesis is potentially a viable cancer therapy.

Several additional commonalities among cancers have been identified, and eventually these may become targets for cancer therapy.⁸⁰ For example, most malignant tumors are metastatic and are able to disseminate cells throughout the body. In breast cancer and malignant melanoma, both of which currently have a poor long-term prognosis in advanced cases, the clinical problem is usually not local control of the tumor. Instead, the problem is that the tumor has metastasized widely at diagnosis and the secondary tumors are themselves able to metastasize. If a treatment could be found to prevent metastasis in a newly diagnosed patient, then curative efforts could focus on treatment of the established tumors and probably provide better clinical success.

Metastatic tumor cells differ from normal cells in that they are able to degrade extracellular matrix. If the ability of metastatic tumor cells to degrade matrix could be blocked, then tumor cells would literally be imprisoned in a cage of matrix they could no longer digest. In human melanoma cell lines, treatment with retinoic acid (vitamin A₁) results in a reduction of expressed levels of collagenase, an enzyme that degrades matrix. Retinoic acid inhibits the ability of melanoma cells to invade through basement membrane, which might make these cells unable to metastasize. However, tumor cells secrete more than a half-dozen other protein-digesting enzymes, including such potent enzymes as acid phosphatase and cathepsin. It may be necessary to block several different enzymes simultaneously, in order to prevent a cell from metastasizing.

Alternatively, it might be possible to interfere with the ability of metastatic cells to "home" to the sites in which they preferentially grow. When melanoma cells metastasize to the lung, they bind specifically to laminin, a molecule that is a component of basement membrane. When laminin molecules in the lung are exposed to an antibody, the laminin is coated with protein so that tumor cells can no longer bind specifically to laminin. Antibodies to laminin inhibit the attachment of mouse melanoma cells to laminin in cell culture, and they also

decrease the ability of tumor cells to metastasize to the lung in the living animal. If it were possible to use a drug to interfere with cell binding to laminin, this might block the ability of melanoma cells to metastasize to lung.

Tumor cells also differ from normal cells in that they fail to undergo terminal differentiation. But if tumor cells could be induced to differentiate, they might cease to grow. There is some evidence that leukemia cells represent a kind of arrested cell development. Certain drugs (e.g., interferon and various derivatives of retinoic acid) have been shown to induce differentiation of leukemia cells in culture, and retinoic acid has even been shown to cause some cell differentiation in acute myelogenous leukemia. Another agent that has been used to induce experimental cell differentiation is cytosine arabinoside (Ara-C), a drug that is widely used in combination chemotherapy for acute myelogenous leukemia. Studies with cultured leukemia cells show that Ara-C induces leukemia cells to differentiate when given at a lower dose than is currently used in chemotherapy. Preleukemic patients frequently develop full-blown leukemia and are also very vulnerable to infection, but a third of the patients treated with Ara-C had a complete or partial remission of their disease. This suggests that induced differentiation may be a viable therapeutic strategy in certain kinds of cancer.

Finally, it might become possible to modulate the responsiveness of tumor cells to growth factors. For example, it might be possible to arrest the growth of tumor cells by making them more sensitive to the growth inhibitory signals conveyed by growth factors. There are preliminary indications that retinoic acid may be capable of increasing tumor cell responsiveness to growth hormones in a way that opposes unbridled tumor cell growth.

RADIATION THERAPY

Radiation therapy differs profoundly from chemotherapy in that radiotherapy can be used only for local control of a tumor, whereas chemotherapy has the potential to treat disseminated or systemic cancer. While it is certainly possible to treat patients with whole-body radiation, which might be a treatment for systemic cancer, toxicity to the bone marrow and intestinal mucosa normally precludes this as a viable treatment option. However, radiotherapy can be highly effective at obtaining local control; about 70% of all cancer patients receive radiotherapy at some time during their treatment. Tumors have been classified into three categories: radioresistant tumors, such as sarcomas,

melanomas, and gastrointestinal tumors; radiosensitive tumors, such as lymphomas; and intermediate tumors, such as adenocarcinomas and squamous carcinomas.

Radiation usually kills cells indirectly, by causing the formation of activated and highly cytotoxic molecules called free radicals. The initial targets of radiation are molecules of oxygen within the tissue, or the water molecule itself, which makes up about 80% of most cells. Radiation exposure causes oxygen or water molecules to ionize and form various free radicals, which then can react with (oxidize) a wide range of other molecules within the cell.

Evidence that DNA is the critical target of radiation damage has come from many separate observations. It has been noted that increased radiation sensitivity is seen in cells from patients with DNA repair deficiencies, such as xeroderma pigmentosum, and that the extent of damage repair deficiency correlates well with disease severity. Experiments have shown that the cell nucleus is 100-fold more sensitive to radiation than the cell cytoplasm, and that chemicals that incorporate into and destabilize DNA act as radiosensitizers. Generally, DNA damage is detected at the same dose level as is required for cell killing, whereas damage to cell proteins occurs only at radiation doses 10-fold higher than used in the clinic.⁸¹

Numerous factors affect the proportion of cells killed by a given dose of radiation.⁸¹ Tissue oxygenation is critical because oxygen is an important source of free radicals, so well-oxygenated tissues are about threefold more radiosensitive than hypoxic tissues. The growth rate of cells in the target tissue is important because cells are more vulnerable to radiation if they are in mitosis (M phase) or G₂ phase, while they are fairly radioresistant in S phase. Thus, a tissue that is growing rapidly, in which many cells are cycling through M or G₂ phase, will be more radiosensitive than a tissue where most of the cells are in G₀ or G₁ phase. Radiation dose rate is a critical variable because if a certain dose is given at a low dose rate, then cells are more likely to be able to repair cell damage and continue growing. Finally, the type of radiation exposure is critical, since different radiation sources have different effects on living tissue. For a given total radiation dose, greater cell kill is achieved with particle beam therapy, using neutrons or protons, than with standard radiotherapy using X rays or gamma rays. Furthermore, for both X rays and gamma rays, total cell kill is determined by the state of oxygenation of irradiated tissue, while neutron radiation is much less dependent on oxygen to achieve cell kill.

There can be complicated interactions between the various factors that affect radiation cell kill. For example, therapeutic radiation is often adminis-

tered in multiple small dose fractions. Fractionated radiation is more successful than single-dose radiation at sterilizing a tumor without damaging normal tissues, because of subtle interactions among the factors affecting radiation cell kill. Normal cells appear to have more capacity for DNA damage repair than most tumor cells, so normal tissues are better able to recover from one small radiation exposure before they are subjected to the next radiation dose. Normal cells that are inadvertently exposed to radiation during the course of tumor treatment are able to repair the radiation damage while tumor cells are not. This is very important because a certain amount of normal tissue exposure to radiation is unavoidable; radiation fractionation is thus used to reduce normal tissue damage. Furthermore, as tumor cells are killed within the tumor mass, the tumor shrinks and blood flow within the tumor often improves, so that there are fewer hypoxic cells within the tumor mass. As hypoxic tumor cells undergo this process of “reoxygenation,” they become more vulnerable to radiation damage because of the increased amount of cell oxygen. Finally, fractionation also permits cycling cells to redistribute through the various phases of the cell cycle, which increases the probability that some tumor cells will be in the sensitive phases of the cycle (Fig. 35).

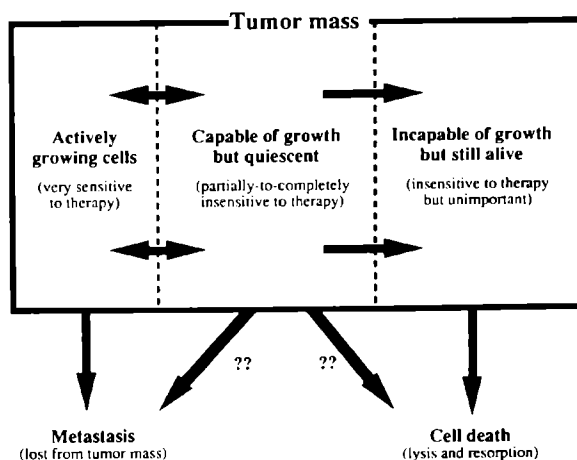


Figure 35. The “ecology” of cells within a tumor mass. Actively growing cells are able to metastasize or remain within the primary tumor and grow; if resources become limiting, these cells may also become quiescent. Quiescent cells may be able to resume growth if resources are no longer limiting, or they may go on to lose their ability to grow. Cells that are incapable of growth nevertheless occupy space within the tumor and consume resources, but they are not of therapeutic importance because they cannot reproduce themselves. These

“nonclonogenic” cells may eventually die because of resource limitations. (From V. T. DeVita, 1987, *Harrison's Principles of Internal Medicine*, edited by E. Braunwald *et al.* Reprinted by permission of McGraw-Hill, Inc.)

Considerable research has focused on how to increase the kill rate of tumor cells while sparing normal cells from radiation effects. Two general approaches have been tried: either the tumor cells are made more sensitive to radiation, or normal cells are protected from radiation. Several chemicals are used today as radiation sensitizers, to increase the rate of tumor cell kill even among hypoxic cells, which are generally radioresistant. Chemicals such as misonidazole or metronidazole diffuse into even poorly perfused or hypoxic cells, where they interact with radiation and potentiate radiation damage to cellular DNA. The alternative approach of protecting normal tissues from radiation has involved two different strategies. A great deal of effort has been spent on improving radiation treatment planning, so that high doses of radiation can be delivered, even to deeply seated tumors, without lethal irradiation of surrounding normal tissues. The other strategy for protecting normal tissues from radiation is chemical radioprotection, which had its origins in the effort to protect soldiers from nuclear radiation in the wars of the future envisioned by the Pentagon. Chemical radioprotectors are often compounds called thiols, which are normally present in cells and which function to protect cells from free radical damage. Thiols are relatively nontoxic and doses of thiol seem to concentrate in normal tissues, not tumor tissues. However, despite some recent successes, both radioprotection and radiosensitization are still experimental techniques.

HYPERTHERMIA

The technique of heating tumor tissue to kill tumor cells is known as hyperthermia. Extremes of heat are not required, since heat in the range of 43 to 45°C is usually sufficient to kill tumor cells (normal body temperature is 37°C). Hyperthermia is capable of killing tumor cells directly, but it can also potentiate the effect of chemotherapy or radiotherapy. Heat tends to kill cells that are in S phase, that phase of the cell cycle most resistant to radiation, so clinical protocols that combine radiotherapy and hyperthermia are becoming increasingly common. Experimental data suggest that hyperthermia inhibits DNA damage-repair by cells after radiation, so that hyperthermia may permit lower total radiation exposures to be used in cancer treatment.⁷²

Tumor cells are more sensitive to hyperthermic cell killing than normal cells, in part because normal cells are better able to repair sublethal cell damage. But cellular sensitivity to heat is also increased by acidic pH, hypoxia, and poor supply of cell nutrients, all of which are conditions associated with

poor tissue perfusion and likely to afflict cells in a tumor mass. Perfusion may be the most critical variable in determining tumor response to hyperthermia, especially since blood flow through tissue is the major way by which heat is dissipated. Poorly perfused tumors are more easily heated to high temperature and cool more slowly after heating is terminated.

Several technical problems thus far limit the clinical application of hyperthermia, and the technique is not yet widely used. Normal tissues surrounding a tumor tend to dissipate the heat quickly, so it can be difficult to maintain an appropriate temperature within the tumor. While it is relatively easy to heat superficial cancers, it can be very difficult to heat deep-seated tumors. This is because of the related problems of depositing heat in the proper tissue volume, while heat is being dissipated through the surrounding tissues. Furthermore, it is very difficult to measure the temperature achieved during hyperthermia, even within a superficial tumor, so it is not yet possible to determine with precision the dose–response relationship between temperature and tumor cell kill. Thus far, cancers of the skin, breast, and eye have shown good responsiveness to hyperthermia, and extensive efforts are being made to apply hyperthermia to other tumors as well.

PHOTODYNAMIC THERAPY

One of the recent additions to the armamentarium of the clinical oncologist is photodynamic therapy (PDT). This treatment is used exclusively to improve local disease control or treat locally recurrent disease. PDT depends on an interaction between laser light and a photosensitizing drug that is nontoxic until it is activated by light. The most commonly used photosensitizer is called Photofrin II, which is usually administered to the patient 24 to 48 hours before laser therapy.

Photosensitizer chemicals are usually members of a class of chemicals called porphyrins, which are unusual in that they localize specifically to tumors. Porphyrins are taken up by most cells, but for unknown reasons they are retained longer by malignant cells. After a period of time, porphyrins are cleared from most normal cells, but are not cleared from tumor cells, so the porphyrin concentration in tumor cells can be much higher than in normal cells. Cells with an elevated concentration of photosensitizer absorb laser energy more efficiently than other cells, and light absorption probably results in the production of free radicals within tumor cells.

Experiments with PDT therapy show that PDT can be very effective against skin tumors or tumors in the lung, since the bronchial tubes of the lung can be exposed to light of the appropriate wavelength using an endoscope. PDT is relatively nontoxic, but it has the drawback that the patient cannot go into normal sunlight for several days after administration of the photosensitizer, or else the patient will experience extremely severe sunburn. PDT is also limited in that the only cells that die are those that are exposed to both photosensitizer and laser light. If tumor cells can avoid exposure to either of these agents, they will be resistant to PDT therapy. However, it is likely that PDT will prove clinically useful in the treatment of cancers of the skin, esophagus, ovary, breast, and bladder.

HORMONE THERAPY

Hormones are bioactive molecules that have a strong effect on certain hormonally responsive tissues, and they can also have an effect on the rate of synthesis of other hormones within the body. Hormone effects within the body are often complex, subtle, and difficult to predict without extensive experimentation. Steroid hormones probably affect gene regulation at the level of synthesis of protein, so application of steroid hormones to the cancer patient can interfere with the ability of tumor cells to manufacture new proteins or to regulate cell proteins at appropriate levels. The steroid hormones include androgens (male hormones), estrogens and progestins (female hormones), and glucocorticoids (hormones that affect glucose metabolism and are generally useful as anti-inflammatory agents). Hormone therapy is often used for cancers of the prostate, uterus, ovary, and breast.

There are two general ways of altering hormone levels in the patient to obtain a therapeutic result (Fig. 36). Hormones can be administered to the patient in additive therapy, or the natural source of hormone within the body can be blocked or removed in ablative therapy. Additive therapy involves the injection of biologically active hormone into the patient, although the particular hormone injected depends on the type of cancer. Appropriate hormone therapy can block progression of cancer and, under the right circumstances, can even induce regression of primary or metastatic tumors. In cases where the primary tumor is inoperable, hormone therapy may represent the best clinical choice for treatment, and can result in significant enhancement of quality and quantity of life for the patient.

Hormone	Effects	Therapy	
		Additive (hormone injected)	Ablative (source removed)
Androgens testes adrenals	Stimulates mitosis in sex organs Induces development of sex characteristics Maintains sex characteristics Increases growth of certain tumors	Androsterone injection (seldom used for breast cancer)	Castration Adrenalectomy (for prostate cancer)
Estrogens ovary placenta testes adrenals	Induces development of secondary sex characteristics Maintains sex characteristics Stimulates growth and maturation of long bones Suppresses ovulation and lactation Increases growth of certain tumors	DES (estrogen) injection (for prostate & breast cancers)	Ovariectomy Tamoxifen (anti-estrogen) Adrenalectomy (for breast cancer)
Progestins ovary placenta	Stimulates ovulation, lactation, & normal growth of uterus Opposes estrogen	High-dose progestin (for breast cancer) Other progestin-like agents (for ovarian & endometrial cancers)	Usually not done
Glucocorticoids adrenals	Induces glucose storage Reduces inflammation	Various injectable steroids (for leukemia, breast, & prostate cancers)	Not done

Figure 36. Summary diagram of the principles of additive and ablative hormone therapy.

The availability of reliable tests for the expression of estrogen receptor (ER) and progesterone receptor (PR) in breast tumor tissue represents a major advance in treatment of breast cancer patients. Tumors with ER expression have a 50–60% response rate to hormone therapy, while those with no ER expression have only a 5–10% hormone response rate. Breast cancer patient prognosis is related to ER expression levels in tumor tissue, and tumors with a high level of ER have a significantly better prognosis. Tamoxifen is a potent antiestrogen, which binds to and blocks the ER expressed in tumor cells, so that tumor cells no longer bind estrogen. Tamoxifen is recommended for the postmenopausal breast cancer patient who is node-positive and ER-positive. A World Health Organization study that enrolled more than 16,000 breast cancer patients was used to test the effectiveness of tamoxifen as adjuvant therapy. Tamoxifen led to a 16% reduction in mortality in postmenopausal women, but only a 2% reduction in mortality in premenopausal women. Tamoxifen thus appears to be

a very effective way to block tumor growth in postmenopausal patients whose tumors are estrogen-dependent.

Ablative therapy for cancer involves the removal or destruction of the source organ for a particular hormone. Thus, ablation of the testes (castration) by surgery or radiation may be used to remove the source of androgens in certain advanced prostate cancer patients. Similarly, surgical removal of the ovaries (ovariectomy or oophorectomy) may be recommended for premenopausal women with advanced breast cancer. However, removal of the testes or ovaries does not remove all sources of steroid hormones, so more extensive surgical procedures may be undertaken under certain circumstances. Ablation of the adrenal gland (adrenalectomy) or the pituitary gland (hypophysectomy), a small gland at the base of the brain, may be done to obtain therapeutic benefit, although these procedures are done less frequently now than in the past. Surgical, chemical, or radiation ablation of a hormone-producing organ necessarily results in the absence of all hormones produced by that organ, so hormone replacement therapy is usually required for patients who undergo extensive ablative procedures.

In hormone-dependent tumors with a positive receptor status, hormone therapy is generally regarded as the appropriate follow-up therapy after surgery. Hormones are also used in the treatment of symptoms of certain cancers, even if the cancer cannot be cured by hormone therapy. For example, steroids may be used to reduce intracranial pressure in brain tumor patients, and hormones can be used to increase appetite in patients suffering from cachexia. Hormones have also been used to control blood levels of calcium in certain patients who become hypercalcemic.

BIOLOGIC THERAPY

Biologic therapy is a new approach to cancer therapy that has become established only in the last several years. It is still too soon to determine whether it will achieve any lasting successes in cancer treatment; only a few hundred patients have been treated by all of the different biologic therapies combined, while literally millions of patients have been treated with chemotherapy or radiation. Thus, it would seem that biologic therapy does not warrant discussion here, since it is far too soon to determine whether it will ever become the therapy of choice for a particular cancer. However, biologic therapy may have the potential to be more selectively toxic to cancer cells than any other

modality. It is quite likely that biologic therapy represents the future of cancer therapy.

Biologic therapy can be defined as cancer therapy that acts primarily through the natural defense mechanisms of the patient, or by the administration of substances that naturally occur in the patient.⁸² Biologic therapy has benefited enormously from recent advances in molecular biology and biotechnology, which have made it possible to obtain a wide variety of biological materials in large quantity for the first time. Biologic therapy can be based on manipulation or modulation of the immune system, in a process called immunotherapy, or on the introduction of new genes into cells, in a process called gene therapy. Modulation of the immune system is now better established than gene therapy in the treatment of cancer, but both therapeutic approaches are evolving so rapidly that it is impossible to predict which will achieve more success against cancer in the future.

Most current efforts at immunotherapy of cancer have taken an approach called adoptive immunotherapy. Adoptive immunotherapy is defined as injection into the patient of immune-active agents, including activated cells of the immune system, which directly or indirectly stimulate an immune response.⁸² Until recently, obstacles to adoptive immunotherapy were the inability to identify cells with specific reactivity against tumors, and the inability to obtain these cells in large quantity for treatment. But now several cell types have been identified that can cause tumor regression in some patients, and these cells can be raised in large quantity in cell culture. The most potent immune-active cells identified thus far are lymphokine-activated killer (LAK) cells and tumor-infiltrating lymphocyte (TIL) cells, both of which are activated T cells. The difficulty of obtaining these cells in large quantity has largely been overcome with the discovery of a hormone that stimulates growth of activated T cells in culture. This hormone, originally called T-cell growth factor, is now known as interleukin-2 (IL-2).

LAK cells are capable of lysing fresh tumor cells in cell culture, but do not lyse fresh normal cells in culture. LAK cells are produced by treating freshly harvested tumor-specific T cells with very high doses of IL-2. In fact, LAK cells are generated exclusively when T cells in culture are exposed to IL-2 at levels far higher than are ever found in the human body. LAK cells apparently do not react to specific tumor antigens, but they are nonetheless able to lyse transformed cells specifically, through an unknown mechanism. Injected LAK cells have proven effective against several experimental tumors in mice,

including microscopic metastases of malignant melanoma in the liver and lungs.

Immunotherapy with LAK cells involves injection of cultured LAK cells into the patient with simultaneous injection of IL-2. The latter is injected so that the LAK cells receive a continual growth stimulation even after injection into the patient. Thus far, nearly 200 patients with advanced and presumably incurable cancers of various types have been treated by Dr. Steven Rosenberg at the National Cancer Institute in Bethesda.⁸² A complete or partial response to treatment was reported in 57% of patients with non-Hodgkin's lymphoma, in 35% of patients with renal cell (kidney) cancer, in 21% of patients with melanoma, and in 13% of patients with colorectal carcinoma. About 10% of patients with advanced melanoma or renal cell cancer have experienced a complete remission of their cancer, a finding that is particularly striking given that both of these cancers are almost invariably fatal when advanced. Recently, some success has been reported in treating patients with IL-2 alone, without simultaneous injection of LAK cells, so IL-2 is evidently able to stimulate the immune response of the patient in some cases. With IL-2 alone, a complete or partial response was reported in 18% of patients with advanced renal cancer, and in 24% of patients with advanced melanoma. Thus, in melanoma, IL-2 alone is as effective in causing tumor regression as is IL-2 plus LAK cells.

TILs are cells that are harvested from the tumor itself after the tumor is surgically excised. Those lymphocytes that have infiltrated the tumor are harvested and induced to grow in cell culture, so that their number is greatly increased. TIL cells generally have a more specific reactivity against tumor cells than do LAK cells and they may be capable of recognizing antigens unique to the tumor. Expanded clones of TIL cells can even be genetically modified in an attempt to further increase their reactivity against a particular tumor. Studies of TIL cells isolated from tumor-bearing mice have shown that TIL cells are 50 to 100 times more potent than LAK cells in treating tumors metastatic to the lung. Immunotherapy of patients with TIL cells is so new as a therapy that it has been tested in only about 50 advanced melanoma patients. However, of the first 50 patients, 38% responded to TIL cell treatment with a partial remission of their cancer. Approximately one-third of advanced melanoma patients have TIL cells with specific activity against their tumor, while two-thirds of patients lack tumor-reactive TIL cells.

The ability to introduce new genes into cells has opened new possibilities for the treatment of cancer.⁸² A small number of patients have thus far been

treated with TIL cells genetically modified to contain a gene that was intended simply as a marker of the injected TIL cells. These studies showed that a new gene could be inserted into the TIL cell, that the new gene could be expressed, and that the general properties of the TIL cell were not lost. Genetically engineered TIL cells have now been detected in the circulation of a patient up to 189 days after cell injection, and modified TIL cells have been found within the tumor mass at 64 days after treatment. This suggests that it should be feasible to genetically modify TIL cells in a more significant way, so as to enhance TIL cell reactivity against the tumor, and to have these modified cells survive in the patient for an appreciable period of time.

The most recent efforts at gene therapy in humans have been directed toward inserting the gene for tumor necrosis factor (TNF) into TIL cells.⁸² TNF is a hormone that can induce regression of a wide variety of mouse tumors when injected at high concentration into the blood of tumor-bearing animals. However, humans are unable to tolerate high systemic doses of TNF, so no successes have thus far been reported in clinical trials of TNF against human cancer. The effort to insert the TNF gene into TIL cells was stimulated by the idea that TIL cells might express the TNF gene within the tumor itself. This could result in a high concentration of TNF in the tumor, without exposing the patient to high systemic doses of the hormone. So far, four advanced melanoma patients have been treated with TNF-secreting TIL cells, but it is still too early to tell whether there will be a clinical response by the tumor.

Gene therapy in the future could include a range of possibilities. It has been suggested that the tumor cells themselves might be genetically modified to make them more antigenic. If newly antigenic tumor cells were then injected back into the patient, they might stimulate a generalized immune response to the tumor. This would amount to immunizing the patient against the tumor after the tumor has already become established. It is unclear whether this approach to gene therapy will be successful.

DEVELOPMENT OF NEW TREATMENT PROTOCOLS

For the past 30 years the National Cancer Institute has been involved in the preclinical testing of new drugs for their anticancer potential. More than 10,000 chemicals a year are now screened for activity against rodent tumors. Chemicals that show activity against rodent tumors, or against human tumor cells in culture, may be selected for further testing to determine their effectiveness

against cancer in humans. Drugs are selected for further testing only if they are effective in the initial screening protocols, and they are carefully validated before they are approved for routine use in the clinic. The procedure used to validate new cancer drugs is a carefully defined clinical trial, with each phase of the trial designed to answer a different question. A clinical trial proceeds from Phase I to Phase IV, in a process that can take years to complete. A Phase I trial is designed to determine the answer to several different questions: the maximum dose of a drug that can be tolerated by the patient; the normal organ toxicity of the drug which limits the maximum possible dose level; the degree of severity of normal organ toxicity; the optimal dose schedule for drug therapy; and the pharmacokinetics of the drug in the bloodstream. In addition, any therapeutic effects of the drug on the tumor will be recorded, although drug efficacy is not yet the focus of the trial. Once these critical questions have been satisfactorily answered, the drug may move into Phase II clinical trials. Phase II trials are designed to determine the therapeutic effects of the drug against a series of different tumor types, and to determine the degree of normal organ toxicity in relation to the therapeutic efficacy of the drug. Phase III trials are designed to compare the therapeutic effects of the new drug on a particular cancer with the therapeutic effects of established treatments for the same cancer. In addition, the toxicity of the new drug is compared with the toxicity of the old treatment, to determine which therapy has the better ratio of benefit to toxicity. Finally, once a drug has gone through all of these separate trials, it will enter Phase IV trials, in which the new therapy is integrated with existing therapies, to maximize tumor response while minimizing toxicity from the combined therapy regimen.⁷⁵

Although many new anticancer drugs are in clinical trials at any given time, many of the new treatment protocols for cancer are actually combinations of existing therapy modalities. Drugs may be combined with each other, or with alternative forms of therapy such as radiation, hyperthermia, or immunotherapy. Furthermore, old drugs may be used in new ways, or for cancers that were not originally treated with that drug.

THE PHILOSOPHY OF CANCER THERAPY

Many physicians, and almost all of the lay public, believe that cancer is incurable and that aggressive therapy, with its associated morbidity and mortality, is pointless. But most practicing oncologists have seen cases where

treatment of seemingly hopeless cancer resulted in either a cure or a very significant extension of patient survival with a reasonable quality of life. For this reason, the current philosophy at most oncology centers is that cancer should be treated aggressively, and that the patient deserves a chance for a cure, however remote that chance may be.

But the desire to treat a cancer aggressively must be balanced against the likelihood of achieving success, and the potential harm to the patient of trying to achieve success. Ethical considerations enter into this balance, and the practicing oncologist is often put into the position of being unable to justify a potentially curative therapy because the likelihood of success is so small. If treatment of a particular cancer involved amputation of a limb, and if the amputation virtually guaranteed cure, then any physician and almost any patient would accept loss of the limb as a necessary evil. But as the likelihood of cancer cure decreases, the willingness of the patient to accept limb loss necessarily declines. At some point a balance is reached where the patient may be unwilling to undergo therapy since the probability of success is low, while there is a certainty of a reduced quality of life following therapy. The advice offered by the physician at this point must be a carefully considered balance between the desire to offer the patient a cure, and the desire to “first do no harm.” We believe that a fully informed patient is better equipped to make the difficult choices that often face the cancer patient.

CHAPTER 10

The Leukemias

OCCURRENCE

Blood is an exceedingly complex mixture of different cell types suspended in plasma. Blood is considered to be the largest organ of the body, and the bloodstream of an adult contains 24 trillion (2.4×10^{13}) red blood cells, or roughly a third of all of the cells in the entire body.⁸³ Yet red blood cells represent only 42–47% of the total blood volume, with the remainder of the blood volume composed of plasma and numerous other types of cells. Red blood cells have a finite and rather short life span, living on average only about 4 months. Thus, red blood cells must be replenished at the rate of about 200 billion (2.0×10^{11}) cells per day. Other types of cells in the bloodstream are present in lesser abundance than red blood cells, but they have an even shorter average life span; certain blood cells called granulocytes survive for only a few hours. To totally replace all of the blood cells that die in a single day, the bone marrow and lymph glands must produce 500 billion (5.0×10^{11}) new cells each day, a mind-numbingly large figure. The required rate of blood cell replacement clearly indicates how vitally important it is that the blood be continually renewed at a normal rate.

The leukemias are a varied group of malignancies that affect the hematopoietic (blood-forming) cells of the bone marrow and lymph tissue. The leukemias, lymphomas, and myelomas all affect hematopoietic cells, but the leukemias are distinctly different from the lymphomas and myelomas. Both lymphomas and myelomas arise as solid tumor masses, whereas the leukemias are composed of individual cancer cells disseminated throughout the bloodstream. Since leukemia cells do not form solid tumors until late in the course of the disease, leukemias are in essence “liquid” tumors. Leukemia cells arise from malignant cells in the hematopoietic tissues of the body, which can produce and release an enormous number of white blood cells (leukocytes) into

the bloodstream. The production of large numbers of abnormal leukocytes interferes with, and even suppresses, the normal processes of blood formation and immune system function. Ultimately, leukemia cells in the bloodstream can infiltrate into various tissues, often forming solid tumors in the infiltrated tissue during late stages of the disease.

Leukemias are traditionally described as either acute or chronic, depending on the clinical course of the disease. In the absence of treatment, acute leukemia is a rapidly progressing disease, which can lead to the death of the patient in only a few months. Chronic leukemia progresses much more slowly, even without treatment, and chronic leukemia patients may survive for many years. However, in the past few decades, acute leukemia has become a curable disease, whereas chronic leukemia has not, so the traditional names have lost some of their original meaning.

Leukemias are also described as being either myelogenous or lymphocytic, depending on the cellular origin of the abnormal leukocytes (white blood cells) (Fig. 37). Myelogenous leukemia is characterized by uncontrolled proliferation of hematopoietic cells located primarily in the bone marrow. This results in the production of large numbers of immature blood cells in the myeloid series, which includes cells called neutrophils, eosinophils, and basophils. (Myelogenous leukemia has also been called granulocytic leukemia, myeloid leukemia, myelocytic leukemia, or myelogenic leukemia.) Lymphocytic leukemia is characterized by uncontrolled proliferation of hematopoietic cells in lymph tissue located in the lymph nodes, spleen, lungs, or occasionally the bone marrow. This results in the production of large numbers of immature cells in the lymphocytic series, which includes blood cells called B cells and T cells. (Lymphocytic leukemia has also been called lymphoid leukemia or lymphatic leukemia.)

There are four main types of leukemia that will be discussed here, with countless subdivisions possible among these main types. However, for the sake of simplicity, we will limit our discussion to the main types, which are acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), and chronic myelogenous leukemia (CML). Together, these leukemias accounted for nearly 28,000 new cancer cases in the United States in 1990, making leukemia one of the moderately common cancers. In 1990, 3% of all new cancer cases were leukemias, and leukemia was the tenth most common cancer in the United States. Roughly half of all leukemias are chronic and half are acute, and the incidence of both acute and chronic leukemias is somewhat higher in men than in women.

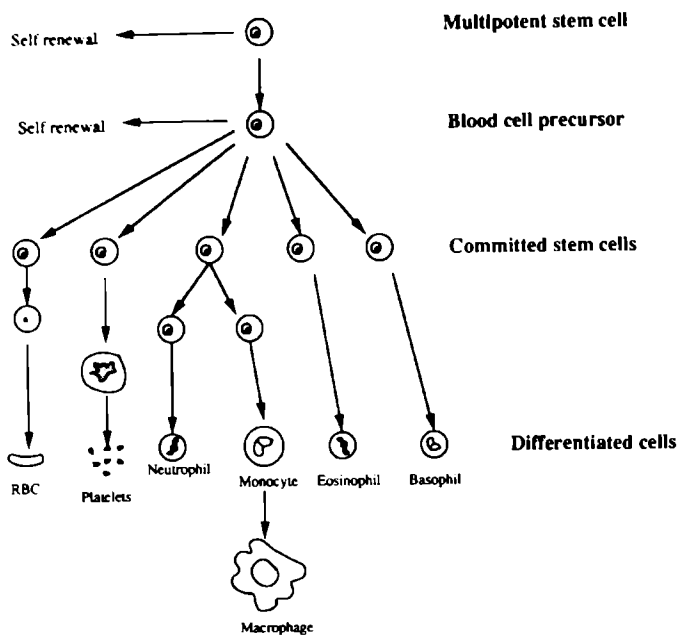


Figure 37. Summary diagram of normal hematopoiesis, showing a proposed genealogy of blood cells. The multipotent stem cell is capable of producing cells that can be either hematopoietic stem cells or lymphopoietic stem cells (this diagram shows hematopoiesis only). The blood cell precursor can give rise to a range of different blood cells, depending on which developmental pathway the daughter cells take. Once a differentiated cell is produced, these cells are no longer capable of generating cells of other lineages. (From J. H. Jandl, 1991, *Blood: Pathophysiology*. Reprinted by permission of Blackwell Scientific Publications.)

ALL is primarily a disease of children and young adults, whereas all of the other leukemias increase in incidence with increasing age. The highest incidence of ALL is in patients under 5 years old, with the disease becoming extremely rare in patients over 30 years old. ALL has a predominance in male children, for unknown reasons. Diagnosis of ALL is made on the basis of the form and immunologic type of abnormal cells in the bloodstream.

CLL tends to occur exclusively in the elderly, and very few individuals under 40 years old are afflicted with this cancer. More than 90% of patients with CLL are over 50 years old, and men are afflicted twice as frequently as women. In older patients, CLL is the most common form of leukemia, and it is more than twice as common as CML in patients over 60 years old. For unknown reasons, CLL is quite rare in persons of Oriental extraction.

AML has a very low incidence in patients under 10 years old, and a moderate incidence in patients from 20 to 40 years old, but disease incidence increases rather sharply after 40 years of age. In patients over 60 years of age, AML is the second most prevalent type of leukemia, with incidence peaking at about 70 years of age. The incidence of AML is about equal in men and women. It is often difficult to distinguish an undifferentiated form of AML from certain types of ALL on the basis of blood cell morphology alone, but the recent availability of antibodies specific to the various types of blood cell has simplified diagnosis.

CML is the rarest of the major forms of leukemia, accounting for only about 15% of all leukemias. CML is slightly more common in males than females, and is relatively rare in patients under 40 years of age. The incidence of CML plateaus at about 60 years of age, when it is the third most common form of leukemia (ahead of ALL). The incidence of CML is equal in males and females after 60 years of age.

The nomenclature, or system of naming, of leukemias can assume a Byzantine complexity, since oncologists name the leukemia after the particular cell type that has undergone the clonal expansion of malignancy. For example, there are seven major subtypes of AML, with names that give little indication that the leukemias are of an acute myelogenous type: acute undifferentiated leukemia; AML with differentiation; promyelocytic leukemia; acute myelomonocytic leukemia; acute monocytic leukemia; acute erythroleukemia; and acute megakaryocytic leukemia. These names are, of course, helpful to the oncologist in describing the cancer, and in conveying a great deal of information to other oncologists in an efficient verbal shorthand, but the names can be overwhelmingly complicated for the patient.

RISK FACTORS

The causes of leukemia in most patients are unknown, although there are indications that both genetic and environmental factors can be important.⁸⁴ There is a high concordance in twins, meaning that if one member of a set of twins develops leukemia, especially in the first year of life, the second member is also likely to contract the disease. Families with an excessive incidence of CLL have been identified, suggesting a strong genetic component to disease development. Furthermore, leukemia often develops in association with a number of different hereditary medical conditions. For example, congenital

disorders in which there is an increased incidence of chromosomal instability, including Fanconi's anemia and Bloom's syndrome, are associated with a 100- to 200-fold higher than normal incidence of acute leukemia. Similarly, patients with Down's or Klinefelter's syndrome, in which there is an aneuploid number of chromosomes, have an abnormally high incidence of leukemia.

Patients suffering from Wiscott-Aldrich syndrome, a congenital deficiency of the immune system affecting male children, are also more likely to develop leukemia. Wiscott-Aldrich syndrome causes eczema, intestinal bleeding, production of a tarry stool, a reduced number of platelets circulating in the blood (thrombocytopenia), and susceptibility to recurrent bacterial infections. Children with Wiscott-Aldrich syndrome usually die before reaching adulthood, either from severe hemorrhage or from overwhelming infection, but about 10% of these patients also develop leukemia. While the defects in the immune system of patients with Wiscott-Aldrich syndrome have been carefully described, the nature of the underlying genetic defect is unknown.

Several studies of the epidemiology of leukemia show that leukemias can cluster within communities or even in successive occupants of the same house.⁸⁵ However, the bulk of evidence suggests that the common forms of acute and chronic leukemia are not contagious. Most studies show that persons in close contact with leukemia patients are no more prone to leukemia than the population at large. The clear exception to this generalization is a rather rare form of cancer called adult T-cell leukemia (ATL), which appears to be caused by a virus known as human T-cell leukemia-lymphoma virus, or HTLV-I. There is evidence that the virus can pass from infected to uninfected individuals, and there are scattered pockets of high viral incidence in coastal areas of Japan, in the Caribbean, and in regions of central Africa. The HTLV-I virus is transmitted largely by breast feeding, sexual intercourse, blood transfusions, or needle sharing, so the virus is not highly contagious. Although laboratory studies have shown that HTLV-I can immortalize certain human white blood cells, the mechanism by which the virus induces cancer is unknown. The latency period between viral infection and development of leukemia may be several decades, suggesting that viral exposure alone is not sufficient to induce leukemia. Additional modifications of cellular DNA may be necessary for leukemia to develop, in a stepwise progression to malignancy. Despite the clear association between HTLV-I and ATL, no viral cause has yet been found for the more common acute or chronic leukemias.

Several environmental causes of leukemia have been described, but the most important carcinogen predisposing for the development of leukemia is

radiation. A clear understanding of the relationship between radiation exposure and leukemia incidence has been obtained from study of survivors of the atom bomb blasts in Japan during the Second World War. The relative risk of dying from leukemia was increased nearly 15-fold among bomb survivors exposed to 2 Gray or more radiation, and disease incidence is dose-related. The incidence of acute leukemia (ALL and AML) increased sharply after radiation exposure, as did the incidence of CML, yet the incidence of CLL did not change. Leukemia incidence reached a peak only 5 to 7 years after exposure, which is a very short period of time for cancer induction. The relative mortality from all other cancers combined, or from cancers at other specific sites, was elevated only about 2-fold with respect to an unirradiated group of people. This suggests that, for unknown reasons, hematopoietic cells are more prone to malignant transformation after radiation exposure than are other cells of the body.

Therapeutic exposure to radiation has also been linked to an increased incidence of leukemia. Patients who received therapeutic radiation to the spine, for treatment of ankylosing spondylitis (arthritis of the spine), were found to develop both aplastic anemia and leukemia in a significant number of cases. Therapeutic radiation primarily increased the relative risk of AML, with minor increases in ALL and CML risk, and no increase at all in risk of CLL. Appearance of excess leukemias began as soon as 2 years after first exposure, and disease incidence crested 5 years after therapy. Radiation-induced leukemia is a significant clinical problem for the cancer patient, since about 70% of all cancer patients receive radiotherapy at some point during their treatment. Thus, treatment of a primary cancer can itself be carcinogenic, and can conceivably induce a second primary malignancy. While the risk of cancer induction from radiation therapy alone is thought to be low, combinations of radiation and chemotherapy have been linked to an increased incidence of AML and solid tumors in the radiation field. Several studies have shown that Hodgkin's lymphoma patients treated with both radiation and chemotherapy have a 5–10% increase in risk of developing AML within 10 years of treatment.⁸⁶

In the United States, about 80% of all exposure to man-made sources of ionizing radiation comes from medical X rays.⁸⁷ The relationship between cancer and the low-dose radiation used for diagnostic purposes is controversial, but there is compelling evidence that diagnostic irradiation of the trunk is associated with increased risk of CML. The risk of contracting CML increases with increasing X-ray dose to active bone marrow. There are also preliminary data suggesting that risk of AML is increased following X rays, and that the

length of time between exposure and development of leukemia is only a few years. At least 16% of all leukemias in the United States are probably caused by diagnostic radiation,⁸⁷ an alarmingly high figure considering that use of diagnostic radiation is increasing.

Certain chemicals have been shown to increase the relative risk of developing leukemia. The best documented leukemogenic chemical is benzene, especially in the case of chronic occupational exposure. There is also evidence that cancer patients exposed to alkylating chemotherapeutic agents, even without radiation, are at a higher risk of developing AML. But risk of leukemia in breast cancer patients treated with chemotherapy is elevated only about 0.5%, and untreated progression of breast cancer is clearly far worse than a small increase in relative risk of leukemia.

NATURAL HISTORY OF THE DISEASE

Both myelogenous and lymphocytic leukemias result from malignant transformation of hematopoietic "stem cells," which are undifferentiated cells that normally can mature into many different types of blood cells. The presence of leukemia cells in the bone marrow or lymph tissue tends to suppress normal hematopoiesis, which causes the most common symptoms of leukemia. Thus, symptoms such as anemia (a reduction in the number of red cells in blood), fatigue, persistent infection, and hemorrhage are common to all of the leukemias.

The leukemias are thought to result from malignant transformation of a single hematopoietic stem cell. Stem cell transformation leads to uncontrolled proliferation of the transformed cell, to form large numbers of leukemia cells in the bone marrow or other organs. If the transformed cell is in the myeloid series, then clonal expansion of the precursor cell leads to AML or CML, whereas if the transformed cell is lymphoid, then ALL or CLL results. Leukemia cells do not mature into normal fully differentiated cells, but rather form precursor cells that fail to mature. The failure of these cells to mature to a nondividing, fully differentiated form results in accumulation of huge numbers of leukemia cells in the bloodstream and bone marrow.⁸⁵

ALL is a malignancy arising from the transformation and clonal expansion of a lymphopoietic stem cell, which is usually located in the bone marrow. Malignant lymphocytes initially remain in the marrow, but the cells eventually emerge and can flood into the bloodstream. About 80% of all ALLs result from

transformed B cell precursors, while the remainder are transformed T cell precursors. ALL has a peak incidence between 2 and 6 years of age, but there is also a gradual rise in incidence in adulthood. The initial spread of ALL is usually asymptomatic, even when marrow replacement leads to serious problems such as anemia. Fatigue, fever, and a general feeling of illness or malaise are common complaints at first presentation, and infants are usually irritable. About 30% of patients present with painful bones and joints, and tenderness of the spine, hips, and sternum are also common, reflecting infiltration of leukemia cells into the marrow cavities of these bones. Painless swelling of lymph glands and enlargement of the spleen are also prevalent, but seldom flagrant.

AML also arises from stem cell transformation, although in this case the stem cell is in the myeloid series. AML results in massive accumulations of myeloblasts in the bone marrow, which eventually cause normal marrow cells to be overwhelmed or dislodged. There is a progressive reduction of red blood cells, platelets, or neutrophils in the blood stream, resulting in anemia, thrombocytopenia, or neutropenia, respectively. This leads to symptoms of fatigue, general malaise, and unexplained but profound weakening over a period of 2 to 3 months. Bleeding can be a major problem in these patients because of an abnormal reduction of blood platelets (thrombocytopenia), which results in an inability to properly clot blood. This causes easy bruising and petechiae (hemorrhagic spots under the skin), as well as chronic bleeding gums, gastrointestinal bleeding, or prolonged bleeding after a minor wound. The relentless overproduction of leukemia cells causes these cells to spill into the bloodstream and infiltrate the spleen, liver, lymph glands, and most other vital organs. Fever and night sweats are often present, but seldom is there weight loss or anorexia. The more obvious diagnostic signs include skin pallor, enlarged spleen (50% of patients), swollen lymph nodes (33% of patients), signs of increased blood volume, and purpura (multiple hemorrhages resembling bruises) in the skin, mucus membranes, and internal organs.

Chronic infection is a frequent complication of acute leukemia, because of a reduction in the number of granulocytes circulating in the bloodstream (granulocytopenia). The risk of infection increases as the number of granulocytes in the bloodstream falls, resulting in persistent infections. Infections can affect the skin or lungs, or the mucus membranes of the mouth, anus, and urinary tract. In more advanced cases of leukemia, granulocytopenia can result in septicemia, an overwhelming systemic infection caused by multiplication of bacteria in the bloodstream. The inability to fight off opportunistic infection

may result not only from the depression in total number of granulocytes, but also from the replacement of normal granulocytes with abnormal ineffective granulocytes derived from leukemia cells. Since chemotherapeutic treatment of leukemia often results in further depression of the immune system, or can damage mucus membranes and clear the way for bacterial invasion, the inability of the leukemia patient to fight opportunistic infections is a major clinical problem.

Enlargement of the liver and spleen (hepatomegaly and splenomegaly, respectively) can result from infiltration of leukemia cells into these organs. Such symptoms are present in 50 to 75% of ALL patients and a small proportion of AML patients. In advanced cancers, involvement of the visceral organs can produce abdominal fullness or bloat, nausea, and appetite depression. Lymph node involvement is more common in ALL than in AML. Infiltration of leukemia cells into the skin, lung, eye, nose, pharynx, and kidneys is common, and males with ALL are likely to have cell infiltration into the testicles as well. Soft masses of leukemia cells, called chloromas, can develop at almost any location. An expanding mass of malignant cells in the bones causes bone pain and tenderness of the sternum in about half of all patients with acute leukemia, particularly in patients with ALL. Kidney abnormalities may develop because of leukemia cell infiltration, or because of blockage of the ureters by enlarged lymph glands. Leukemia cells can also infiltrate into the space around the brain, causing meningitis, or there can be actual infiltration of the brain or spinal cord. Neurologic involvement is relatively rare at first diagnosis, but the brain is a common site of relapse, particularly in patients with ALL. Patients with acute leukemia can also develop certain metabolic abnormalities. For example, hyponatremia (a reduced concentration of sodium ions in the blood) and hypokalemia (a reduced concentration of potassium ions in the blood) are relatively common, because of impaired kidney function. Hyperuricemia (an increased concentration of uric acid in the blood) can result from an accelerated death rate of blood cells, since dying cells release a chemical precursor of uric acid.

The natural history of the chronic leukemias is somewhat different from the acute leukemias, in that the chronic leukemias are initially rather asymptomatic. In more than 25% of patients with CLL, and in 20% of patients with CML, the disease is diagnosed as an incidental finding from a blood test. In the early stages the only symptoms noted by the patient are characteristic of anemia. However, there is a convergence of symptoms in acute and chronic leukemia, so that symptoms of advanced disease are rather similar in both acute

and chronic leukemia. After diagnosis, the most significant difference between acute and chronic leukemia is in the rate of disease progression; in acute leukemia, the whole course of the disease can occur within a few months if left untreated, whereas in chronic leukemia disease progression can take years.

CLL is a disease that also begins with the transformation and clonal expansion of a hematopoietic stem cell, except that in this cancer the stem cell is of the lymphoid line, usually a B cell precursor. The earliest symptoms of CLL are fatigue and reduced exercise tolerance, which worsens progressively as the number of abnormal lymphocytes in the blood increases. Within a few years of the first sense of enervation, other symptoms become manifest, including swollen lymph nodes, enlargement of the spleen and liver, and a relentless accumulation of lymphocytes in the bloodstream and infiltrating into tissues. Nodal enlargement can cause obstruction of the upper respiratory tract, and there may also be infiltration into the lungs, skin, central nervous system, and gastrointestinal tract. Enlargement of the spleen occurs early in over 90% of patients, and as the disease progresses the spleen may enlarge progressively. Marrow infiltration can eventually result in marrow failure, with resultant thrombocytopenia and neutropenia, and the associated conditions of persistent bleeding and chronic infection. CLL is second only to the AIDS virus as a cause of acquired immunodeficiency, and CLL patients are prone to a wide range of opportunistic infections and secondary malignancies. The most frequent secondary cancers are solid tumors of the colon or skin, but multiple myeloma and B cell lymphomas occur at least ten times more frequently in CLL patients than in the population at large.

CML is a disease originating from the clonal proliferation of a stem cell that overproduces granulocytes and their precursors. These cells may appear normal when viewed under the microscope, but they are functionally defective. CML follows a typical course consisting of a long preliminary “chronic phase,” when the patient suffers from a progressive increase in the number of defective granulocytes in the bloodstream. This is followed by an “acute transformation” phase, during which increasingly immature granulocytes are produced, and eventually the disease culminates in a “blast crisis,” in which enormous numbers of functionless blast cells are produced. Symptoms during the chronic phase of the disease are generally more subtle than the symptoms of acute leukemia, but can include fatigue and diminished exercise tolerance of several months’ duration. In addition, there may be anorexia and weight loss, and splenomegaly is seen in more than half of all CML patients. The extent of splenomegaly can be used as a gauge of the duration of the chronic phase, as

very large spleens herald acute transformation of the disease. Acute transformation is an accelerated phase of the disease, in which the enhanced growth of myeloid blasts usurps marrow space and causes anemia, thrombocytopenia, and neutropenia. This phase usually occurs about 3½ years after initial diagnosis, but it can occur within weeks or it may be delayed 20 years. Acute transformation is the prelude to a complete arrest of myeloid cell differentiation, and results in a "blast crisis" that is generally fatal within 3 months. Blast crisis in CML essentially resembles an accelerated form of AML, which suggests that AML patients are presenting to the physician in blast crisis. Blast crisis leads to extensive thrombocytopenic bleeding, marrow failure, and overwhelming opportunistic infection.

BIOLOGY SPECIFIC TO THE TUMOR

AML cells have an average doubling time of 7 days, which is considerably slower than the average doubling time of 18 hours in normal hematopoietic cells. Thus, AML, like the other leukemias and lymphomas, is not a disease caused by too-rapid cell proliferation, but rather is a disease of abnormal cellular development. Leukemia cells are generally unresponsive to those growth factors that modulate growth and maturation of normal cells. After stem cells divide, the daughter cells would ordinarily go through a complex process of differentiation as they develop into mature blood cells. Leukemia occurs when these immature cells fail to mature, so that they are never able to assume their normal function yet they are able to continue dividing. Malignant stem cells typically produce a large number of abnormal daughter cells, so leukemia is characterized by an overabundance of one or a few cell types in the blood.

Once leukemia cells form masses, the population doubling time of the tumor mass can be as much as ten times as long as the cell doubling time, indicating that there is a substantial rate of cell loss from the tumor. Nevertheless, leukemia cells can crowd out normal marrow cells and cause a reduced production of normal functional blood cells. It has been calculated that AML is lethal when the number of tumor cells is 5×10^{12} cells (about 1.5 kg), a tumor cell burden that amounts to roughly 20% of the cells in the bloodstream. This number of tumor cells is generally accumulated within 40 to 80 doubling times, over a period ranging from 2 to 8 years.

A great deal of research has shown an association between leukemia and various chromosomal defects. It is much easier to study chromosomal defects

in leukemia cells than in other tumor cells, because of the ease of sampling leukemia cells from the bloodstream. Thus, more than 80% of the research on chromosomal mutation in cancer has been devoted to leukemia, even though leukemia represents fewer than 5% of the new cancer cases each year. Leukemia is usually associated with an acquired chromosomal defect that causes abnormal cell maturation and permits a clone of mutant cells to overgrow its neighbors. This chromosomal defect often results in activation of a particular oncogene, by bringing that oncogene into a new chromosomal location where it is regulated differently. Since oncogenes often code for growth factors or growth factor receptors, activation of these genes can free leukemia cells from the constraints that limit growth of normal cells.

Perhaps the best known example of a specific chromosomal defect in leukemia is the so-called “Philadelphia chromosome,” which is seen in more than 95% of patients with CML (Fig. 2). This defect, first described in 1960, was one of the earliest and strongest pieces of evidence that chromosomal aberrations can cause cancer. Chromosomal translocation brings together two genes that form an unusual gene product, called *c-abl/bcr*, which appears to play a role in development of CML. The *c-abl/bcr* gene product acts as a growth factor, but the mechanism by which this growth factor induces clonal proliferation of the affected stem cell is unknown. In any case, the Philadelphia chromosome is seen throughout the course of CML, both in remission and in relapse, and it is generally unaffected by treatment.

Virtually 100% of patients with acute promyelocytic leukemia, a subtype of AML, show another type of reciprocal translocation, in which a piece of chromosome 17 is exchanged for a piece of chromosome 15. The cellular oncogene *c-erbA* is near the break point on chromosome 17, so the *c-erbA* oncogene is transposed to the end of chromosome 15, where the gene product of the *c-erbA* oncogene can be overexpressed. The exact mechanism by which regulation of the *c-erbA* oncogene is lost is still unknown, but it is clear that this loss of regulation is leukemogenic. In addition to these chromosomal translocations, there are other chromosomal defects, from point mutations to chromosomal loss, which are associated with leukemogenesis.

Independent mutations to both alleles of the p53 tumor suppressor gene are a frequent finding in T-ALL cell lines and in the cells of some T-ALL patients in relapse. To determine whether human tumor cell growth could be suppressed by the p53 gene, a T-ALL cell line that lacked the normal p53 gene was transfected with a recombinant virus that carried a functional p53 gene. Expression of a normal p53 gene in the tumor cells reduced the tumor cell

growth rate in culture, suppressed the ability of cells to form colonies in culture, and blocked the ability of these cells to form tumors in mice. This suggests that suppression of acute leukemia cells via transfection with a wild-type p53 gene may be a viable therapeutic strategy for T-cell ALL.⁸⁸

Recent laboratory results suggest that it may eventually become possible to reconstitute a normal hematopoietic system in leukemia patients.⁸⁹ A line of mice, called severe combined immunodeficient (SCID) mice, was used for this research because normal mice with a competent immune system easily reject transplanted human cells. Human bone marrow was injected into the SCID mice, at the same time as the mice were treated with massive doses of human growth factors. These growth factors permitted human bone marrow cells to survive in the mice for up to 4 months. In addition, the human marrow cells grew within the marrow spaces of the mouse and gave rise to a wide range of fully differentiated human blood cells. If SCID mice were treated with human cell growth factors, the stem cells produced fully differentiated human blood cells of multiple myeloid and lymphoid lineages. If human erythropoietin was also given to the SCID mice, the mice even produced human red blood cells. The bone marrow of mice that received human growth factors contained both human hematopoietic stem cells and partially differentiated human myeloid and erythroid cells. Mice that received injected human cells, but that were not treated with human growth factors, had few human cells in their marrow at the end of the experiment, and these few cells did not form as broad a range of different mature cell types. Thus, it is possible to inject a mouse with human marrow cells and artificially reconstitute virtually the entire hematopoietic system of a human.⁸⁹ If this knowledge can be transferred to humans, it may eventually become possible to reconstitute a normal hematopoietic system in a leukemia patient. This would probably require use of radiation to wipe out the cancerous hematopoietic system of the patient, before reconstituting a normal hematopoietic system using compatible stem cells and massive doses of human growth factors.

CHAPTER 11

Lymphomas and Myelomas

OCCURRENCE

Both the lymphomas and the myelomas are properly regarded as cancers of the immune system. Since several different types of leukemia can also affect cells of the immune system, the distinction between leukemias and lymphomas may seem subtle, but in practice the difference is fairly straightforward. Leukemias typically do not form palpable solid tumors until fairly late in the course of the disease, whereas lymphomas usually have already formed solid tumors by the time they are diagnosed. As well as this obvious practical difference, there are differences between leukemias and lymphomas in terms of which immune cell type is transformed to undergo clonal expansion.

In the past, lymphomas were described as either Hodgkin's or non-Hodgkin's lymphoma, with few distinctions made among the non-Hodgkin's lymphomas. This was largely a reflection of the fact that distinguishing between Hodgkin's and non-Hodgkin's lymphoma was easy, whereas virtually any other distinction among the non-Hodgkin's lymphomas was exceedingly difficult. The non-Hodgkin's lymphomas are a confusing array of different tumors that arise from any of a number of different clonal malignant cells. However, the advent of sophisticated diagnostic techniques has made it easier to distinguish among the various non-Hodgkin's lymphomas, so it is now possible to delineate subcategories of disease.⁹⁰ Because of this newfound diagnostic precision, there is now a trend toward defining the non-Hodgkin's lymphomas as lymphocytic lymphomas, with various subtypes defined.

The American Cancer Society has estimated that in 1990 there were a total of about 43,000 newly diagnosed cases of lymphoma, and about 11,800 new cases of multiple myeloma. Taken together, these cancers accounted for more than 5% of all new cancer cases in 1990. Among the 43,000 lymphomas, about 7400 (17%) of the new cases were Hodgkin's lymphoma, while the remaining

35,600 (83%) of the new cases were made up of various lymphocytic lymphomas (LLs). Among the LLs, typically about 80% of new cases are B-cell tumors, 15% are T-cell tumors, and the remaining 5% are tumors derived from macrophages or the cellular descendants of macrophages. For the sake of simplicity, we will only consider B- and T-cell LLs, Hodgkin's disease, and multiple myeloma in this chapter.

Collectively the LLs are the most prevalent cancers of hematopoietic tissue,⁹¹ and LL is the seventh most common cancer overall. Because these cancers typically affect younger persons than do most other cancers, LLs rank fourth among cancers in terms of person-years of life lost. The mean age of the LL patient population is only 42 years old, and the age-specific incidence of LL climbs steeply from about age 20. In adults, LLs are about 50% more frequent in males than in females, as is also the case with several forms of leukemia. The higher incidence in males begins at such a young age that it cannot be explained by occupational exposure to carcinogens.

Hodgkin's disease (HD) also affects nearly half again as many men as women, with an average age at diagnosis of only 32 years.⁹² This cancer is unusual in that it does not show a steadily rising incidence with age, but rather there are two separate peaks of incidence with increasing age. One peak of incidence occurs in persons 25–30 years of age, and the second peak is found in individuals 75–85 years of age. Disease incidence in persons 45–50 years old is roughly half that in persons 25–30 years old. This very unusual age distribution, combined with the fact that disease incidence increases so abruptly between 15 and 30 years of age, has suggested to some scientists that young-adult HD may result from an infectious disease or a deranged immune response to an infectious disease. HD has undergone one of the largest declines in mortality of all cancers in the period between 1973 and 1987, and this has been coupled with a substantial decline in disease incidence: mortality from HD declined 49.5%, while disease incidence declined 15.9% over the 14-year period.⁹³

Multiple myeloma (MM), or more simply myeloma, is a solid tumor derived from clonal proliferation of a fairly well-differentiated immune system cell, so MM has more in common with the lymphomas than with the leukemias. In MM a plasma cell, whose normal role is to secrete antibodies, is transformed and secretes massive quantities of protein antibody directed against an often irrelevant antigen. MM is primarily a disease of the elderly and incidence steadily increases with age. The median age at diagnosis is 64 years and the disease is rare in patients under age 40. Disease incidence is remarkably similar in many different countries, and males are only slightly more likely to get MM

than females. However, blacks have a disease incidence nearly twice as high as whites.

RISK FACTORS

Both the lymphomas and the myelomas are thought to result, in part, from chronic stimulation of the immune system (Fig. 38). Many factors can lead to immune system stimulation and many factors have been implicated in lymphoma causation. However, perhaps the most important risk factor for lymph-

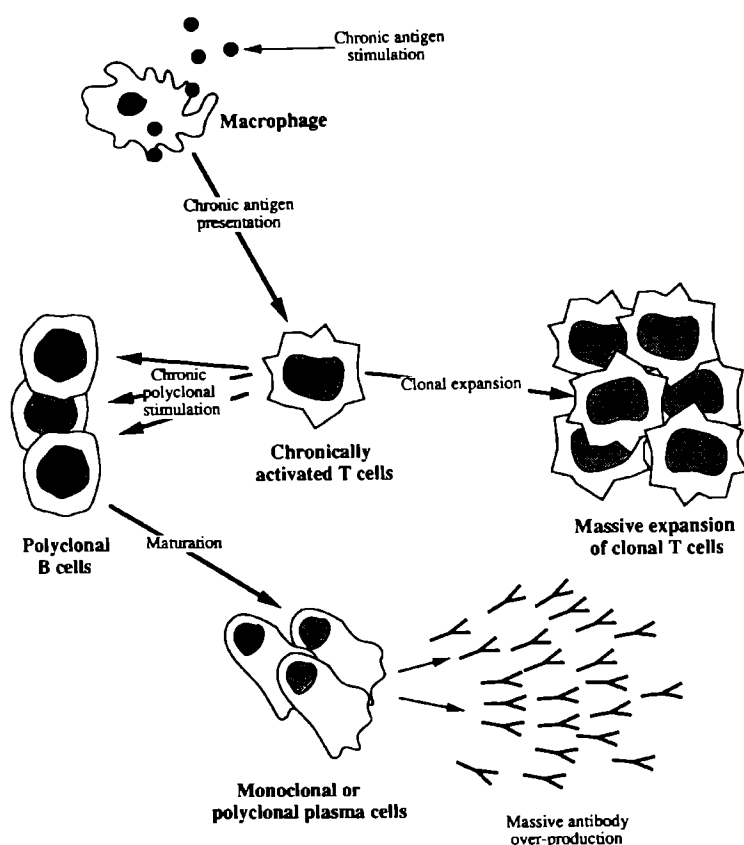


Figure 38. Diagram of hypothetical events during chronic immune system stimulation. These events may be involved in the generation of immune system cancers such as a lymphoma or myeloma. For comparison with events during normal immune system stimulation, see Fig. 28. (From J. H. Jandl, 1991, *Blood: Pathophysiology*. Reprinted by permission of Blackwell Scientific Publications.)

phoma and myeloma is chronic viral infection. Many of the viruses that have been implicated in human cancer causation can stimulate cell proliferation and may also have mutagenic effects on host cell DNA. This would seem a likely scenario to produce cancer, yet the actual molecular mechanism of cancer causation by viruses is unknown. What is clear is that none of the relevant viruses alone can cause cancer development; additional modifications of the host cell genome are required, in a stepwise progression to malignancy.⁹⁴

There is now very clear evidence that viruses can cause lymphoma in various animals, including rodents, birds, cats, and cows. Recently, evidence was obtained that another virus, called human T-cell leukemia-lymphoma virus, or HTLV-I, can cause human lymphoma. This virus has been linked to an unusual form of LL, called mycosis fungoides (or cutaneous T-cell lymphoma), and to adult T-cell leukemia-lymphoma. This type of leukemia-lymphoma is very rare in the United States, but it is prevalent in coastal areas of Japan, and among blacks in the Caribbean and parts of central Africa. Antibodies to HTLV-I are present in more than one million people in Japan, so exposure to the virus is widespread, but only about 500 patients a year develop the associated form of cancer in Japan. Furthermore, the latency period between infection and disease development can be many years. Both the high level of viral exposure in Japan and the long disease latency confirm that viral exposure alone is not sufficient for cancer development.

There is now reason to suspect that several other human lymphomas may also be viral in origin. A virus similar to HTLV has been isolated from cells of a human B-cell lymphoma. While the HTLV viruses identified thus far affect T cells exclusively, a distantly related virus, called bovine lymphoma-leukemia virus, causes a B-cell malignancy in cattle. A virus may also be responsible for HD, since the immune defect that accompanies HD affects T cells and thus could result from infection with a T-cell-specific virus. Finally, there is evidence that infection with the Epstein-Barr virus (EBV) is associated with Burkitt's lymphoma, and perhaps also with HD and B-cell lymphoma. EBV infection in adolescence causes infectious mononucleosis, and long-term study of patients with mononucleosis shows that these patients are at a slightly elevated risk of developing lymphoma. Antibodies directed against EBV are found in blood serum of patients with Burkitt's lymphoma, and copies of the viral genome are actually present in Burkitt's tumor cells.

There is ample evidence of an increase in cancer incidence in organ transplant patients who are immunosuppressed to prevent organ rejection.⁹⁵ Patients who receive a kidney transplant and are chronically immunosuppressed are 20- to 50-fold more likely to develop cancer, with lymphoma risk

specifically increased 35-fold over normal. There has also been a striking increase in the incidence of highly malignant, undifferentiated lymphoma among young people suffering immune system suppression as a result of infection with the AIDS virus. This highly malignant form of lymphoma was originally diagnosed almost exclusively in middle-aged adults. The genome of the AIDS virus is not expressed in cancer cells, indicating that tumors develop as an indirect effect of the immunosuppressed state. However, it is unknown whether lymphomas are opportunistic, occurring simply because immune surveillance is impaired, or whether AIDS infection actually predisposes to lymphoma in particular. The progression of disease development in immunocompromised AIDS patients seems to be as follows: viral infection first sabotages the T-cell network, then viral particles previously hidden within B cells are released and these particles act as stimulants to B-cell division, and finally there is a chromosomal mutation in a B cell that actually triggers development of lymphoma.

The genes carried by an individual can apparently influence the development of lymphoma. This is suggested by the fact that only a tiny fraction of patients with HTLV-I or EBV exposure ever develop lymphoma. Certain diseases are associated with inheritance of a particular genetic signature in the "major histocompatibility" gene complex. One such disease is Sjogren's syndrome, which is an immunologic disorder characterized by a variety of autoimmune symptoms, including rheumatoid arthritis and progressive destruction of the salivary glands. Nearly 10% of patients with long-standing Sjogren's syndrome develop lymphoma, meaning that the relative risk of lymphoma in Sjogren's patients is elevated more than 1000-fold relative to a control population. The incidence of lymphoma is also elevated in patients with hereditary disorders such as Fanconi's anemia (associated with a high incidence of lymphoma and leukemia) and ataxia-telangiectasia (associated with a low incidence of lymphoma and leukemia). Fanconi's anemia is an inherited instability of the chromosomes resulting in a high frequency of chromosomal rearrangement, which suggests that genetic mutation is ultimately responsible for lymphoma causation. There is also an increase in lymphoma incidence in the families of patients with immunologic disorders, which is consistent with the idea that the human genome influences lymphoma development. For example, one study found an increased incidence of HD in the siblings of HD patients, particularly if both siblings were male.

Lymphoma can also be the end result of progression of another primary malignancy in the cancer patient. Several B-cell cancers, including B-cell lymphoma and MM, occur at least ten times more frequently in patients with

chronic lymphocytic leukemia (CLL) than would be predicted on the basis of chance alone. Poorly differentiated large-cell lymphoma (also called Richter's syndrome) seems, in many cases, to be an endpoint in the progression of CLL. Richter's syndrome occurs when advanced-stage CLL transforms into high-grade lymphoma, which spreads from leukemic lymph nodes into the surrounding organs. This tumor spreads exceedingly rapidly, and can form a coalescent mass of tumor involving the lymph nodes, liver, marrow, and spleen.

The risk factors for HD are poorly understood, but it has been observed that there is an increased incidence of HD in some families, and that HD cases can cluster in schools and neighborhoods. This suggests that HD may actually be an infectious malignancy.⁹² The bimodal pattern of disease incidence, with a sharp peak of incidence in young persons and a second peak in aged persons, suggests infection as a cause of the young-adult form. Furthermore, young and old patients seem to contract different types of HD: the young-adult type is typically either nodular sclerosing HD or lymphocyte-predominant HD, whereas in older patients the most common type of the disease is lymphocyte-depletion HD. In economically advantaged countries, where small families, single-family housing, and freedom from childhood disease are common, there is a tendency for the early peak of HD incidence to affect young adults, whereas in developing countries, where childhood disease is rampant, HD tends to affect children. Finally, it has been observed that 20% of HD patients show evidence of EBV infection. In fact, viral DNA is often found within Reed–Sternberg cells, those cells that are diagnostic of HD. Yet, medical personnel who specialize in care of lymphoma patients do not show an increased incidence of HD. There is relatively little evidence of lateral transmission of HD, which should be found if the disease is in fact a transmissible cancer, and many of the identified clusters of disease incidence could have happened by chance alone.

It is possible that HD is stimulated by viral infection, but that other factors are important in determining which of the virally infected patients will progress to develop HD. Thus, HD may be a rare manifestation of a common infection. Factors that increase the risk of early exposure to infection, such as large family size or crowded living conditions, decrease the risk of contracting HD. In Japan, where living conditions tend to be more crowded than in the United States, the first peak of disease incidence is entirely absent. It may be that viral infection must occur during a particular window of time, perhaps in early adolescence, in order to result in development of HD.

The major risk factors for MM are unknown, but there is evidence to

suggest that myeloma cells can result from chronic activation of the immune system. Once a B cell is activated, it multiplies and differentiates to form a plasma cell, which secretes large quantities of antibody directed against that antigen that first stimulated the immune system. MM results from the clonal growth of a plasma cell, suggesting that chronic stimulation of B-cell proliferation may, under certain circumstances, lead to MM. The hypothesis that abnormal B-cell activation plays a role in induction of MM is consistent with evidence that myeloma in mice develops only after exposure to an antigen that stimulates the immune system. In humans, many myeloma cells secrete antibodies that are specific for commonplace antigens, some of which are proteins normally present in the human body.⁹⁶ This has led to the suggestion that MM is an autoimmune cancer, but this is a remote possibility. Nevertheless, it has been found that patients with MM are slightly more likely to have a particular genetic "signature" (in their major histocompatibility gene complex), known as HLA-B5, which is associated with an increased risk of several autoimmune diseases.⁹⁷ In particular, HLA-B5 is associated with an increased risk of ulcerative colitis (3.8-fold increased risk) and Behcet's disease (6.3-fold increased risk).⁹⁸ Behcet's disease is a very unusual syndrome, affecting primarily young men, in which there are recurrent episodes of ulceration of the oral and genital mucosae, eye inflammation, formation of nodules in the legs, and thrombophlebitis (inflammation of the veins). The superficial similarity between Behcet's disease and very mild symptoms of MM is certainly thought-provoking.

There is no direct evidence for the involvement of oncogenes in MM, and the only well-known risk factor for MM is radiation exposure. Incidence of MM increased in persons exposed to radiation from atom bomb blasts in the Second World War, but only after a 20-year lag period.

NATURAL HISTORY OF THE DISEASE

At one time in the past, all of the non-Hodgkin's lymphomas were categorized as either reticulum cell sarcoma or lymphosarcoma. Recent efforts at classification of the LLs have provided a much more detailed picture of the various subcategories of disease. The newest classification from the National Cancer Institute includes 14 different types of LL, yet nearly 5% of lymphomas are still unclassifiable under this system.⁹¹ The clinical presentation of these various LLs can differ sharply since they span a range from low-grade,

relatively indolent cancers, through intermediate-grade, fairly anaplastic cancers, to high-grade, anaplastic, and very aggressive cancers. At diagnosis, roughly a third of LL cases are low-grade, somewhat less than half are intermediate-grade, and another 15–20% are high-grade. The remaining roughly 10% of cases are considered miscellaneous, either because they affect macrophages rather than B or T cells, because disease presentation is unusual, or because the cancers are unclassifiable as to grade.

Overall, the most common symptoms of LL are painless, localized, or generalized enlargement of lymph nodes, often combined with enlargement of the liver or spleen.⁹⁹ Among the low-grade LLs, symptoms at first presentation may be limited to a painless swelling of one or a few lymph nodes. These cancers may be so free of notable symptoms that they are first diagnosed during the course of a routine physical. Nevertheless, these cancers can be widely disseminated at first presentation, and many are resistant to treatment. The intermediate-grade LLs typically have a more rapid tumor growth rate at presentation and involve more obvious symptoms. There may be a generalized swelling of lymph nodes, complicated by lymph node involvement in the chest around the lungs. Involved nodes can form bulky masses, and there may also be tumors outside the confines of the lymph nodes. Nearly one-third of all intermediate-grade LLs present with metastatic tumor in the tonsils or throat, the lacrimal (tear) glands, the salivary glands, the gastrointestinal tract, the bones, the skin, or the central nervous system. The high-grade LLs are characterized by tumors with an extremely rapid growth rate; for example, small noncleaved cell lymphomas can double in volume in 24 to 48 hours, which makes this the fastest growing of all human cancers.⁹¹ Thus, the symptom most typical of high-grade LLs is a rapidly expanding tumor mass that may or may not be a lymph node.

HD usually presents with one of two sets of symptoms in young people. Either there is an otherwise-asymptomatic enlargement of lymph nodes, to form painless rubbery swellings, or there are symptoms such as fever, night sweats, weight loss, and occasionally itchiness of the swollen lymph nodes. Lymph node swelling (adenopathy) is rather common in young people, and is usually indicative of infection rather than cancer, so this symptom may not be noted until fortuitous diagnosis by a physician during a routine physical examination. Occasionally, swelling of nodes in the chest cavity is discovered after an X ray is taken to diagnose the cause of a dry, nonproductive cough. In older HD patients, the most common symptoms are fever of unknown origin and night sweats, followed by increasing malaise and weight loss. In these

patients there may be no lymph node swelling noted, since the involved nodes are often within the abdomen. The fever of HD is usually intermittent, occasionally following a cyclical pattern called Pel–Ebstein fever, in which days or weeks of fever alternate with periods of normal temperature. Generally, the presence of fever, night sweats, and weight loss indicates a poorer prognosis than simple lymph node swelling. Occasionally, there is also alcohol-induced pain, which can indicate to the physician the best place to biopsy for a definite diagnosis. In advanced cases of HD, tumor cells may invade the spleen, bone marrow, liver, and other organs. As the volume of tumor increases, and if treatment is unsuccessful in arresting disease progress, there can be cachexia (dramatic weight loss), widespread tumor dissemination, and frequent opportunistic infections.

The diagnostic feature of HD is the presence of an unusual cell, called a Reed–Sternberg cell, in the bone marrow or in aspirates of bone marrow cells. In microscopic views of marrow, Reed–Sternberg cells are very large and obvious, and are the key criterion for distinguishing between HD and the various LLs. The origin of these cells is unknown, although there is evidence to suggest that Reed–Sternberg cells may be derived from either B or T cells, or occasionally both.⁹² This would suggest that HD is not a single disease entity, but that there may be subtypes of HD which make it as complicated (and as poorly understood) as the LLs. It has been suggested that HD is actually a group of 4 different malignancies, which show slight differences in the appearance and immune reactivity of the Reed–Sternberg cells, and which also differ in disease manifestation and prognosis.

The remaining major type of lymphoid cancer is MM. The most common symptom of MM is bone pain, which is present in nearly 70% of all MM patients. Bone pain typically involves the back and ribs and, unlike the pain of metastatic carcinoma, which is worst at night, the pain of myeloma is brought on by movement. Bone pain is caused by erosion of the bone surrounding a metastatic tumor nodule, and persistent localized bone pain in an MM patient can indicate the presence of a pathological fracture of bone. Lytic bone lesions result from the proliferation of tumor cells within the bone marrow, and from the release by tumor cells of a growth factor called osteoclast activating factor (OAF). OAF activates osteoclast cells, which are normally involved in bone remodeling or the resorption of bony tissue; OAF release by tumor cells results in the erosion of bone to create new space for the growing tumor cells. Abnormal activation of osteoclasts causes the development of multiple “punched-out” lesions, which are small to large, circular holes that may



Figure 39. X-ray image of the skull (facing to the right) of a 60-year-old male patient who was examined because of complaints of general malaise. The image reveals numerous lytic sites in bone called “punched-out” lesions. These lesions are caused by the release of a growth factor by tumor cells located within the bone marrow. (Photograph courtesy of Dr. Michael Richardson and Dr. James A. Nelson, University of Washington School of Medicine.)

perforate bone and that can occur even in the skull (Fig. 39). This structurally weakens the bone, and also causes an abnormal elevation of calcium ions in the bloodstream. Hypercalcemia is responsible for a number of severe symptoms in advanced MM patients, including lethargy, depression, weakness, and confusion.

MM is also characterized by several other related symptoms that may be present at diagnosis. In about 25% of MM patients, recurrent infection is the presenting symptom, and more than 75% of MM patients will have a serious infection at some point in the course of their disease. The most common secondary infections are pneumonia and pyelonephritis, a bacterial infection of the kidney. Susceptibility to infection arises because MM patients generally have a decreased production and an increased destruction of normal antibodies, or a general depression of the immune system. Kidney failure can affect up to 25% of MM patients, and abnormalities of the kidney are noted in about half of all MM patients. Kidney failure can result from hypercalcemia, recurrent infection, invasion of myeloma cells into the kidney, or abnormal deposition of protein (usually antibodies) in the kidney. Anemia can affect up to 80% of MM patients, as a result of marrow replacement by tumor cells and the inhibition of normal hematopoiesis by growth factors released from myeloma cells. The blood of MM patients can become so viscous that it does not easily perfuse tissue, which causes headache, fatigue, visual disturbance, and damage to the retina.

BIOLOGY SPECIFIC TO THE TUMOR

The confusing array of different lymphocytic lymphomas probably arises because of a cellular equivalent of Murphy's law: for every cell type and stage of differentiation, there exists a cancerous counterpart.⁹¹ Since the process of immune cell differentiation and maturation is exceedingly complex, malignant transformation of the immune cells gives rise to an equally complex array of tumor types. Malignancies of certain relatively well-differentiated cells, such as B or T lymphocytes and various other cells, give rise to low-grade lymphomas that are amenable to treatment. Malignancies of poorly differentiated lymphoid stem cells, such as B- or T-cell precursors called lymphoblasts, give rise to high-grade, very aggressive lymphomas that resist treatment. In general, low-grade lymphomas tend to be follicular (nodular), and composed of relatively small cells, whereas high-grade lymphomas tend to be diffuse, invasive of bone marrow, and composed of large cells. Follicular LLs as a group have a distinctly better prognosis than diffuse lymphomas. This is consistent with the expectation that highly anaplastic tumors, like diffuse lymphoma, grow more rapidly, are more likely to metastasize widely, and are more resistant to treatment.

Several chromosomal rearrangements have been described in LL, some of which suggest that oncogene activation is involved in the progression of lymphoma. One of the best known chromosomal translocations is seen in Burkitt's lymphoma, a B-cell lymphoma that frequently afflicts children in Africa. This tumor typically forms a solid mass in the facial bones or in the abdomen, and it can grow with great rapidity. The geographical distribution of Burkitt's lymphoma suggests that it is virally induced, and that the virus may be spread by biting insects. Chromosomes from Burkitt's lymphoma cells typically show a pattern of rearrangement in which the terminal part of chromosome 8 is exchanged with a part of chromosome 14. This reciprocal translocation brings the *c-myc* oncogene (on chromosome 8) into close proximity with the genes involved in forming antibodies. About 80% of Burkitt's lymphoma patients have tumor cells with this type of translocation, while the remaining 20% of patients show different translocations that also involve the *c-myc* oncogene. The net result is the same: the normally stable *c-myc* oncogene is brought into a chromosomal region that is notoriously unstable. There is loss of regulation of the oncogene, which becomes vulnerable to mutation, and *c-myc* expression can reach very high levels. This results in production of a protein growth factor that by an unknown mechanism, stimulates tumor formation by B

cells. A similar translocation is also occasionally seen in non-Burkitt's lymphomas.

Another reciprocal translocation that is commonly linked to lymphoma is seen in follicular-type LLs. About 85% of lymphomas with a follicular morphology, as well as a third of all diffuse large-cell lymphomas, show a reciprocal translocation between chromosomes 14 and 18.⁹¹ This translocation brings a gene called *bcl-2* (for B-cell leukemia-lymphoma-2), which is located on chromosome 18, into a region on chromosome 14 containing genes involved in antibody production. It is unclear whether *bcl-2* is actually an oncogene, or whether the *bcl-2* protein drives carcinogenesis, but the frequent association between gene translocation and disease expression is suggestive. The existence of the same characteristic translocation in all lymphoma cells in a given patient also supports the idea that LL is clonally derived from a single transformed lymphoid cell. Finally, the break point on chromosome 18 lies near a hereditary fragile site, which supports the possibility that there is a hereditary predisposition to this type of lymphoma.

Understanding of HD is presently limited by our ignorance of the cellular origin of Reed–Sternberg cells. Clearly these cells are cancerous, since they are aneuploid (have an abnormal number of chromosomes) and they are capable of inducing cancer when transplanted into an unaffected animal. Yet several other cell types are also abnormal in HD patients, and it is unknown whether these other cells are intimately involved in the disease process, or whether they only represent an immune response to Reed–Sternberg cells. It is presently believed that HD is a T-cell disorder, and that Reed–Sternberg cells arise from activated T helper cells, but this understanding is evolving. Furthermore, cells that resemble Reed–Sternberg cells can be seen in diseases other than HD, including mycosis fungoides and benign lymphoid hyperplasia.

MM is a malignant proliferation of a plasma cell, the cell whose normal function is to secrete protein antibodies. Antibodies are proteins that are tailored to bind specifically to an antigen that stimulates an immune response, whereas an antigen is any substance that induces a state of sensitivity or resistance to infection. There is a certain amount of circularity in these definitions, since antigens are defined by their ability to induce antibodies, and antibodies are defined by their ability to bind to antigens. Plasma cells must be able to respond to an incredible variety of antigens present in the environment, and must be able to mount an antibody attack upon an antigen even if that antigen has never been encountered before. Thus, plasma cells have become specialized to divide rapidly when exposed to a novel antigen, and to produce

large quantities of antibody at short notice (8 to 14 days). They have also developed remarkable adaptations that permit them to generate an incredible variety of antibodies. The process by which a normal plasma cell synthesizes and secretes an antibody is exceedingly complicated, even involving rearrangements of genes on the chromosomes in order to produce the required diversity of antibodies.

Since MM is a cancer of these highly specialized plasma cells, it is not surprising that transformed myeloma cells continue to secrete antibodies, usually in large quantity, and often directed against irrelevant antigens. For example, some human myeloma cells secrete antibodies directed against molecules in the red blood cell membrane, or in the extracellular space surrounding normal cells. What this means is that myeloma growth may be driven by antigens that are inescapably a part of the normal human body, and that this omnipresent antigen may promote the growth of a mutant B-cell clone. Clearly, this can be very harmful to the patient, since it means that the patient may suffer an autoimmune response directed against the tissues of his or her own body. Even if the aberrant antibodies do not result in a debilitating autoimmune response, they can be present in such large quantity that they are indirectly harmful to the patient.

Chemical analysis of blood serum taken from patients with MM will often show the presence of a protein that is nearly absent in normal patients but is present in high abundance in patients with MM. This protein is called M (for monoclonal) component, and is comprised of numerous identical subunits of an aberrant antibody synthesized by the myeloma cells. Analysis of the quantity of M component in the bloodstream can actually be a fairly accurate indicator of the total tumor burden in the patient. The minimum amount of M component detectable by traditional techniques corresponds to about 10^9 (1,000,000,000) tumor cells. If it is assumed that M component is synthesized equally by all myeloma cells, then the growth rate of the cancer can be calculated from the length of time it takes the concentration of M component in the bloodstream to double.⁹⁶ The average doubling time for M component is about 6 months. Since the number of cell doublings needed to accumulate a clinically significant number of tumor cells (10^{12}) is 40, and since each doubling takes about 6 months, it can be estimated that it would take a single malignant plasma cell about 15 to 20 years to become clinically evident. This may explain why MM becomes increasingly common in older patients. Unfortunately, M component is not unique to MM, so it cannot serve as a disease marker in asymptomatic patients; M components can also be present in patients with chronic lymphocy-

tic leukemia, chronic myelogenous leukemia, breast cancer, colon cancer, and a variety of noncancerous conditions including cirrhosis and parasite infection. Often, aberrant myeloma antibodies can be detected in the urine of MM patients. These urine proteins are called Bence–Jones proteins, and patients may excrete 5 to 10 grams of Bence–Jones protein a day. These proteins can accumulate in the kidney, plugging up the excretory tubules of that organ, and causing chronic kidney inflammation and eventual kidney failure.

Myeloma cells often secrete several proteins in addition to the abnormal antibodies typical of transformed plasma cells. These proteins can be growth factors that stimulate nearby noncancerous cells; an example of this is OAF, which was discussed earlier in this chapter. However, myeloma cells can also secrete growth factors that stimulate growth of other myeloma cells and even of normal (nontransformed) B cells. However, the most damaging consequence of growth factor secretion by myeloma cells are the bone lesions so typical of MM.

CHAPTER 12

Brain Tumors

OCCURRENCE

Cancers of the brain and central nervous system (CNS) account for only about 2% of the new cancer cases diagnosed in the United States each year. Yet brain tumor patients can require such intensive medical care that about 25% of the entire annual cost for care of cancer patients is allocated to these patients. Furthermore, even though only 15,600 patients were diagnosed with primary cancer of the brain in 1990, roughly 25% of all patients with systemic cancer eventually develop cerebral metastases during the course of their disease.

Despite their relative rarity in adults, CNS tumors are the most common solid tumors in children, accounting for 20% of all pediatric cancers. Typically, brain tumors have two peaks of incidence, one in childhood (3–12 years of age) and the other much later in life (50–70 years of age). Pediatric tumors are quite different in histology and behavior from the tumors of adulthood. Nearly two-thirds of all pediatric brain tumors are infratentorial, meaning that they occur in the cerebellum or brain stem, while only one-third of adult brain tumors are infratentorial, with the remainder occurring in the cerebral hemispheres. Males and females are afflicted equally overall, but certain tumors are more common in men, while others are more common in women.

The principal histologic types of brain cancer are meningioma, which represents 12–15% of brain tumor cases, and glioma, which represents about 45% of all brain tumors. Another 25–30% of brain tumors are classified as secondary tumors, metastatic from primary cancers principally of the lung or breast.¹⁰⁰ However, this seeming simplicity is very misleading: within the glioma category are at least five distinct entities, and there are about 10–15% of primary brain tumors that do not fit into the category of either glioma or meningioma. Among the less common brain tumors are: pituitary adenoma, a

grouping comprised of three distinct entities, which together make up less than 5% of brain tumor cases; Schwannoma, a relatively benign tumor of the cells sheathing the nerves, which also make up less than 5% of brain tumors; primitive neuroectodermal tumors (PNETs), which generally predominate in children; and a diverse collection of rare tumors.

This complexity of tumor types arises because of the complexity of brain structures, and the numerous cell types that comprise those structures. The cells in the brain fall into five broad categories: the neurons, or nerve cells themselves; the neuroglia, cells that surround and nourish the nerve cells; the meninges, which are membranes covering the brain and spinal cord; the nerve sheath cells, which provide an insulating or protective covering around the neurons; and certain cells within the brain that appear to be relatively undifferentiated. Since malignant transformation can seemingly affect any one of these cell types, the diversity of possible tumors is rather overwhelming. In the following material, we will limit ourselves principally to a discussion of primary meningioma and glioma, and the discussion will also be limited to tumors of the brain itself and will not cover pediatric tumors or tumors outside the brain.

Meningiomas are tumors of the membranes (meninges) surrounding the brain and spinal cord. These tumors are often relatively benign and tend to proliferate over the brain surface without actually invading into brain. Because of their location at the brain surface, meningiomas are often amenable to surgical excision.

Gliomas are tumors of the neuroglial cells, those cells that appear to be involved in supporting, nourishing, and sustaining neurons.¹⁰¹ The relative frequencies of the five principal types of glioma, in descending order, are: glioblastoma (20–25%); astrocytoma (15–20%); ependymoma (5–6%); medulloblastoma (2–3%); and oligodendroglioma (2–3%). Astrocytoma is a rather general term, which can denote a tumor ranging from benign to highly malignant. The grading of a tumor from benign to malignant is based on cell density, cell growth rate, shape of the cell nucleus, development of blood vessels within the tumor, extent of tumor necrosis, and loss of the specialized structures seen in normal glial cells. An Astrocytoma Grade I or II is often called a benign or low-grade astrocytoma, an Astrocytoma Grade III would be called an anaplastic (malignant) astrocytoma, and an Astrocytoma Grade IV would be called glioblastoma multiforme. The distinction between astrocytoma and glioblastoma is made because the cells in these two tumor types can look strikingly different from each other.

RISK FACTORS

Risk factors for cancers of the brain are poorly understood; although several different risk factors are suspected, only a few are known to contribute to brain cancer. A role for genetics has been fairly well established in brain tumor incidence, because of an association between malignant cancer and several hereditary conditions of benign cell proliferation.

The best-known familial condition that predisposes to malignant brain cancer is called neurofibromatosis, or von Recklinghausen's syndrome. This is the disease that has been called "Elephant Man" syndrome, although it is likely that the famous Elephant Man actually had a different disease. Neurofibromatosis is a rare condition characterized by the appearance of small, pigmented skin lesions, called café au lait spots, in childhood. This is followed at a later age by the appearance of multiple benign nerve tumors (neurofibromas) beneath the skin, running along the course of the peripheral nerves. These neurofibromas are firm nodules, often stalked and protruding from the skin, which are composed of a tangled array of all of the elements of peripheral nerves, including Schwann cells, which normally sheath neurons. These benign proliferations can occur as near-microscopic growths or as huge masses, at virtually any site in the body. Neurofibromatosis can be a very disfiguring condition with potentially fatal consequences, since neurofibromas can occur even within the enclosed space of the cranial vault. In about 5% of neurofibromatosis patients, a benign lesion is transformed into a malignant tumor, usually in the large nerves of the neck or extremities. Moreover, both glioma and meningioma occur more often in patients with neurofibromatosis, and up to 50% of patients with optic glioma also have neurofibromatosis.

Various other hereditary diseases can predispose a patient to malignant brain cancer. For example, tuberous sclerosis is associated with a triad of clinical features, including convulsive seizures, mental deficiency, and characteristic fine, wartlike lesions in a butterfly pattern over the nose and cheeks. These facial adenomas are benign proliferations of connective tissue in the skin. However, tuberous sclerosis is also associated with an increased incidence of retinal tumors and gliomas. Turcot's syndrome, a condition characterized by development of benign colonic polyps, is also associated with an increased incidence of brain tumors and colon cancer. Patients with Osler's disease, a hereditary syndrome resulting in multiple small hemorrhages of blood capillaries in the skin and mucus membranes, are apparently more likely to develop hemangioblastoma, a benign tumor of the cerebellum.

There is no firm evidence linking chemical carcinogenesis to brain tumors in humans, but there is very strong evidence of a role for chemical carcinogenesis of brain tumors in rats. If a pregnant rat is exposed to any of several chemicals called nitrosoureas, a high proportion of the rat's offspring develop brain tumors. Prolonged prenatal exposure can cause virtually a 100% incidence of highly malignant, invasive brain tumors in offspring. In the few human studies done, vinyl chloride and certain chemicals used in the rubber industry have been associated with brain tumors.

There is also no clear evidence for a viral role in induction of human brain tumors, but the experimental evidence is again suggestive. Brain tumors can be induced in beagles by injection of the Rous sarcoma virus into the brain, and the resulting tumor closely resembles human malignant astrocytoma. Brain tumors can also be induced in rats by injection of purified avian sarcoma virus, and can be induced in monkeys by injection of the human polyoma JC virus. The monkey brain tumor was induced after an 18-month delay, but the resulting tumor closely resembled human astrocytoma. If the long delay between viral exposure and tumor induction is also typical in humans, this may explain why there has been no success in isolating viruses from human brain tumors. Nevertheless, patients with primary lymphomas of the brain tend to have a higher incidence of infection with Epstein–Barr virus (EBV). DNA extracted from human tumor tissue, but not from normal tissue, contains EBV viral DNA.

Trauma and X rays have been implicated as causes of meningioma and acoustic neuroma, but this evidence is not compelling. Patients who have received X-ray therapy to the scalp have an increased incidence of brain tumors, and meningiomas may be more common in women who have received repeated medical or dental X rays. There is some evidence suggesting that head trauma can lead to an increased likelihood of tumor at the site of trauma. However, trauma to the CNS may cause hemorrhage of an established tumor, so the trauma may appear to have caused the tumor. Finally, there is some evidence that exposure to high electrical fields can result in an increased incidence of glioma in electricians and high-tension line workers for the telephone company, but this evidence is highly controversial.

There appears to be a clear relationship between immune system suppression and the incidence of primary lymphoma of the brain. Organ transplant recipients, whose immune systems are suppressed to prevent organ rejection, have a 350-fold higher incidence of brain lymphoma. Furthermore, the inci-

dence of CNS lymphoma is also much higher in AIDS patients and in patients with hereditary immune deficiencies.

NATURAL HISTORY OF THE DISEASE

A better understanding of the natural history of brain tumors has been obtained within the last decade, largely because of a dramatic improvement in diagnostic medical imaging of brain tumors. Until the fairly recent past, the imaging method of choice for brain tumor patients was X-ray computed tomography (CT). Virtually all hospitals have CT scanners because of their general utility, and CT can generate medical images in as little as a few seconds. Contrast-enhanced CT images can detect 95% of intracranial masses, although brain abscess, cerebral infarction, multiple sclerosis, and vascular malformations may give an appearance superficially like tumor. CT is exceedingly good at imaging bony structures such as the skull, so it is very useful for determining the extent of calcification within a tumor or the degree of bone erosion around a tumor. However, CT cannot clearly delineate soft structures such as brain, which do not absorb much X-ray energy. Consequently, use of CT is declining for imaging brain tumors and other soft tissue pathologies.

The imaging modality of choice for a suspected brain tumor is currently magnetic resonance imaging (MRI), which can visualize tumors less than 1 cm in diameter without exposing the patient to X rays. MRI is capable of generating very clear images of soft tissue like brain, because MRI is actually imaging the density and physical properties of water within tissue (Fig. 40). Because normal brain has a high water content, brain structures are clearly visualized, and displacement of these structures by tumor is often the clearest indication of a malignancy. However, MRI scanners are very costly, so they are not available in all hospitals.

The most common symptoms of brain tumor are caused by expansion of the tumor in the enclosed space of the cranial vault, resulting in compression of normal brain.¹⁰² Because this can occur with any type of brain tumor, the symptoms of the various brain tumor types are rather similar. But the exact nature and severity of the symptoms caused by compression are dependent on the location of the tumor and the rate of tumor growth. Generally, brain tissue can shift to accommodate the presence of a tumor smaller than about 3 cm, so tumors will usually not be diagnosed until they have passed this threshold size

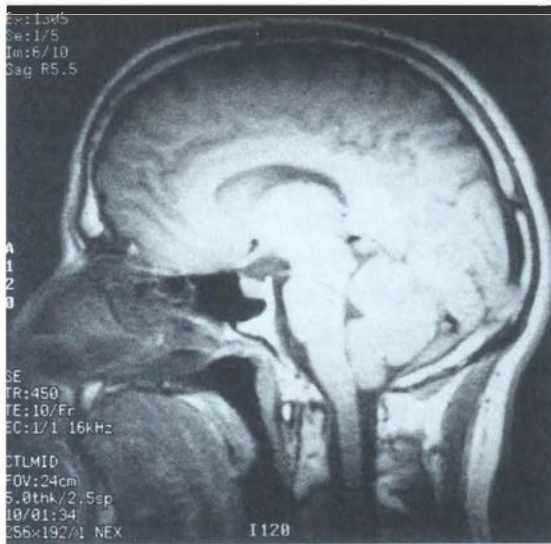


Figure 40. Magnetic resonance imaging (MRI) can be used to generate anatomical images of striking clarity and beauty; this image simply demonstrates normal brain anatomy. The patient is facing to the left, with the nose faintly visible and the nasal membranes apparent behind the nose. The cerebral hemispheres fill most of the volume of the skull, with the furrows (sulci) clearly visible. The spinal cord is seen entering the picture from the bottom right, where it widens to form the medulla oblongata. The cerebellum is visible near the base of the skull, beneath the posterior portion of the cerebrum. (Photograph courtesy of Dr. Kenneth Maravilla and Dr. James A. Nelson, University of Washington School of Medicine.)

(Fig. 41). Once a tumor exceeds 3 cm in diameter, it will begin to compress the brain and its blood supply, or the cavities through which the cerebrospinal fluid flows. At this point the tumor may begin to cause severe symptoms.

Headache is the primary symptom first presented to the physician in most brain tumor patients. In 35% of cases, headache is the first symptom noted, and 71% of patients complain of headache by the time they are diagnosed. Headache results from elevation of intracranial pressure or direct compression of brain structures by the tumor. The headache is often worst in the morning and eases over the course of the day, because fluid drains from the brain more easily when the patient maintains a vertical posture. Although there is often a correlation between the site of the tumor and the origin of the headache, many patients complain of a diffuse headache. Headache may be relieved by standard medications such as aspirin, but usually these headaches are less responsive to medication than prior headaches. Headache may be associated with vomiting, and often the latter will occur without a feeling of nausea.

Another very common symptom of brain tumor is an epileptic seizure. Either adult-onset seizure activity or an increasing severity of previous seizure activity can result from tumor growth. An epileptic seizure is the first symptom noted in 38% of brain tumor patients, and 54% of patients will have a seizure by the time they are diagnosed. Seizure also results from compression of the brain by the tumor, and can take the form of a generalized *grand mal*-type seizure or a

Figure 41. MRI image of a 43-year-old female patient, revealing a large cerebral mass that was later diagnosed as a glioblastoma multiforme. This image clearly shows the tumor as a large bright mass in the posterior part of the cerebrum (lower left). Compare this image with Fig. 18, from the same patient during the same examination: this image was acquired under a protocol designed to suppress the signal from edematous brain around the tumor, whereas Fig. 18 was optimized to show edema around the tumor. Note that brain structures have been displaced to the right because of the large mass of tumor and edematous brain. (Photograph courtesy of Dr. Kenneth Maravilla and Dr. James A. Nelson, University of Washington School of Medicine.)



focal seizure of one of the limbs. Often the first seizure is a momentary loss of concentration or awareness that is not really noted by the patient. Curiously, the presence of seizure as an early presenting sign is often associated with a more favorable prognosis.

Tumors of the frontal part of the brain may attain considerable size before symptoms appear, and often the associated symptoms are rather difficult for the nonspecialist to detect. Subtle mental changes occur as the first symptom in 17% of brain tumor patients, and such changes are present in 52% of patients at diagnosis. These mental changes can take the form of a decline in intellectual ability and an increase in emotional lability, coupled with increased jocularity. Alternatively, there may be slowness of comprehension, loss of acuity in business affairs, memory disorders, or apathy, lethargy, and drowsiness. Spontaneity of thought or action may be lost, or there may be development of urinary incontinence or an uncoordinated gait. Dysphasia, the loss of comprehensible speech, or motor weakness is generally the sign of a fairly advanced tumor.

A number of symptoms are associated with growth of a tumor in a specific focal region of the brain. For example, tumors near the temple may cause personality changes that resemble psychotic thought disorders. There can be a combination of auditory hallucinations, abrupt mood swings, and altered patterns of sleep, appetite, or sexual function. Large tumors involving the surface of the cerebral motor cortex give rise to weakness affecting a certain, often small, portion of the body. Tumors occurring deeper into the motor cortex cause weakness that affects a larger portion of the body, and a small tumor at a critical site can cause paralysis of one whole side of the body. Tumors growing toward the posterior part of the cerebrum are associated with disorders of communication, including aphasia (loss of the ability to communicate by speech, writing, or signs). Tumors of the cerebellum or brain stem can lead to partial paralysis or loss of feeling in those regions of the body served by the cranial nerves, together with loss of limb coordination.

Hemiparesis, a slight paralysis affecting one side of the body only, is present in 10% of brain tumor patients as an initial symptom, but 43% of patients suffer from hemiparesis at diagnosis. Similarly, vomiting is present as an initial symptom in 8% of patients, but 31% of patients show this symptom at diagnosis, whereas impaired consciousness is present as an initial symptom in 5% of patients, but 25% show this symptom at diagnosis. These surprising discrepancies between the initial prevalence of a symptom and the prevalence of that symptom at diagnosis do not mean that brain tumors necessarily progress rapidly. Instead, the discrepancy indicates the degree of impairment that many patients are willing to tolerate before they seek medical help. In fact, visual field impairment may be tolerated for so long by the brain tumor patient that there is virtual blindness at the time of first presentation to the physician. A series of 653 patients with cerebral gliomas were studied, to determine the delay between when a patient first became aware of a brain tumor symptom, and the time of definitive diagnosis by surgery.¹⁰³ It was found that there was a mean delay of 1.65 years between symptom onset and definitive diagnosis, with a wide range of variation possible.

Compression of normal brain by a growing tumor is a major problem, which is aggravated by the buildup of fluid generally associated with brain tumor growth. In normal brain, blood capillaries are surrounded by a tight-knit group of cells forming the blood–brain barrier. These cells isolate the brain from toxic materials that may be present in the bloodstream. But as a brain tumor grows, the blood–brain barrier typically is disrupted and fluid will begin to leak into surrounding brain tissue. This fluid leakage can result in edema, or an abnormally high water content in brain surrounding tumor (Fig. 18). Edema

can cause a whole host of problems, since development of edema within the brain is often associated with elevated cranial fluid pressure. Elevated intracranial pressure can interfere with the flow of blood through the brain, can displace brain structures from their normal position, can compress the cranial nerves as they leave the brain, and can cause impairment of parts of the brain that are not directly invaded by tumor. If the tumor mass becomes large enough to block the normal flow of cerebrospinal fluid within the brain, obstructive hydrocephalus can develop. This is excessive accumulation of fluid within the ventricles of the brain, resulting in dilation of these spaces and greatly elevated intracranial pressure.

The familiar vocabulary of oncologists takes on a somewhat different meaning when applied to the brain tumor patient. The distinction between benign and malignant is much less relevant in brain tumors than in other types of tumor, because many tumors with the histologic features of a benign tumor are still incurable. A tumor that is well differentiated and slow-growing, but surgically inaccessible, will be fatal more rapidly than a malignant tumor in a location amenable to surgery. Tumors of identical histology may have very different prognoses based on their location within the brain and the feasibility of surgical resection. A primary brain tumor that is highly malignant histologically is nevertheless unlikely to metastasize to sites outside the brain. Both benign and malignant tumors can produce profound, irreversible impairment of mental abilities. For these reasons, traditional tumor grading is usually not done for brain tumors, and staging relies more on an evaluation of the general and neurological health of the patient. In fact, the initial clinical approach to benign and malignant brain tumors is identical.

The first major type of brain tumor we will discuss is meningioma, which is typically a nonmalignant tumor. These tumors tend to afflict women more frequently than men, and are frequently found in women with breast cancer, in which case the meningioma may contain estrogen or progesterone receptors. Meningiomas are usually rather slow-growing, and neurological deficits may evolve over many years. Because meningiomas are tumors of the membranes overlying the brain, they are often surgically resectable. But the meningeal membranes are closely adherent to brain tissue, and the membranes penetrate deeply into the brain at the sulci, those characteristic furrows on the brain surface. Meningiomas can even occur on the underside of the brain. For this reason, some meningiomas may be deemed inoperable, and tumor site, rather than tumor histology, is the major determinant of prognosis.

The other major type of brain tumor is glioma, which is usually a highly malignant tumor. Gliomas are subdivided into five different types, the rough

order of increasing malignancy being oligodendroglioma, ependymoma, medulloblastoma, astrocytoma, and glioblastoma. Oligodendrogliomas occur infrequently and are often benign in histology and extremely slow-growing. These tumors can form microscopic calcifications that are easy to visualize by X rays. Typically these tumors are located in the frontal lobe of the brain, or within the ventricles, so surgical access may be good. If a tumor is a pure oligodendroglioma with a benign histology, surgery may grant a good long-term prognosis.

Ependymomas are also glial tumors, which arise from ependymal cells lining the ventricles through which cerebrospinal fluid flows. Ependymomas can arise from ependymal cells in any portion of the brain, but about 70% occur in the fourth ventricle, beneath the cerebellum. Even a small ependymoma can obstruct the flow of cerebrospinal fluid, causing hydrocephalus. Ependymomas tend to occur in childhood or adolescence, which can make radiotherapy or chemotherapy problematic because of toxicity to the developing brain. The anatomic location makes total surgical excision very difficult, regardless of the degree of malignancy.

Medulloblastomas are malignant tumors of the glia which are more common in childhood: about 25% of childhood brain tumors are medulloblastomas, and only 25% of medulloblastomas occur in patients over age 20. These tumors are often referred to as primitive neuroectodermal tumors (PNETs) because they are composed of cells that seem able to differentiate into a wide variety of cell types. All PNETs share a propensity for local invasion, dissemination through the ventricles of the brain, and metastasis to various sites outside the brain, including lung, liver, spinal column, and pelvis. Yet medulloblastomas often respond rather well to treatment, and they typically grow in the lower portion of the cerebellum, so surgical access may be good.

Low-grade astrocytomas (Grade I and II) occur throughout the brain and spinal cord. In children and young adults they tend to develop in the optic nerves, cerebellum, and brain stem, whereas in adults they tend to develop in deeper parts of the cerebrum. These tumors can be associated with neurofibromatosis or tuberous sclerosis, and about 20% of patients undergoing surgery for control of chronic seizure disorders are found to have low-grade astrocytomas. These tumors tend to grow slowly over the course of several years, and the tumor may consist of a small nodule of tumorous cells and a large fluid-filled cyst. An Astrocytoma Grade I is composed of cells that are more densely packed than in normal brain, but these cells differ little in appearance from normal glial cells. Such a tumor can be difficult to distinguish from

astrocytosis, a benign hyperplasia of astrocytes that is often associated with self-repair of the brain near degenerative lesions or local inflammations. In Astrocytoma Grade II, there is an increased number of blood vessels supplying the tumor, and an increase in the degree of pleomorphism (variability) of tumor cell nuclei. Low-grade astrocytomas produce symptoms by displacement of normal brain structures or invasion of nerve tracts in the brain. This slow-growing tumor can change to a more malignant form, which results in rapid tumor progression, with clear evidence of growth in medical images, and development of edema around the tumor.

Anaplastic astrocytomas (Astrocytoma Grade III) are highly malignant glial tumors with a more anaplastic pattern of cell growth than is seen in low-grade astrocytomas. In Astrocytoma Grade III there is an increase in the proportion of dividing cells and an increase in the number of cells with dark-stained or hyperchromatic nuclei. Anaplastic astrocytomas can arise almost anywhere within the brain, although they occur more frequently in the cerebrum. Whereas astrocytomas grow at an increasing rate as the tumor grade becomes higher, anaplastic astrocytomas are still rather slow-growing. However, they are diffusely invasive of surrounding tissue, sending out thin tendrils of cells into the brain. If surgery is attempted, these tendrils are usually left behind and can cause tumor recurrence rate to be very high.

Glioblastoma multiforme (Astrocytoma Grade IV) differs from Astrocytoma Grade III in having a greater degree of cellular anaplasia, or variability in cell size and shape. Typically, tumors designated as glioblastoma multiforme will show necrosis within the tumor mass, with extensive hemorrhage and the formation of large fluid-filled cysts. There may be giant tumor cells scattered among more normal-appearing cells, and the capillaries within the tumor often have an unusual form, as if they had been transformed to a cancerous state by the presence of the tumor. Glioblastomas are similar to anaplastic astrocytoma in being diffusely invasive of normal brain, so tumor cells are usually left behind during surgery. For this reason, surgery alone is not expected to result in cure of either anaplastic astrocytoma or glioblastoma.

BIOLOGY SPECIFIC TO THE TUMOR

The blood–brain barrier is composed of cells that form a sheath around the blood capillaries, which insulates cells of the brain from direct contact with blood-borne materials, so that any chemical moving from the blood to the brain

must be transported across the endothelial cells that form the barrier. Since endothelial cells are selective as to which chemicals they will pass across to the brain cells, neurons are isolated from many chemicals in the bloodstream. The existence of this barrier is very important to the metabolism of normal brain cells, since virtually the only metabolic substrate transported across the blood–brain barrier is glucose. But the blood–brain barrier is also very important in considering the biology of tumor cells within the brain.

An intact blood–brain barrier can isolate tumor cells from the chemotherapeutic agents intended to kill them. This can lead to a physiological resistance of tumor cells to chemotherapy, even though the tumor cells may have no inherent cellular resistance to the drug. This is particularly true if the chemotherapeutic agent is water-soluble, because a water-soluble drug must stay in solution in the blood and cannot diffuse across the blood–brain barrier. If a drug used for chemotherapy is fat-soluble, it can dissolve through the membranes of the endothelial cells in the blood–brain barrier, and so gain access to the tumor even if the blood–brain barrier is intact. This is why carmustine (BCNU) is so commonly used for the treatment of brain tumors: BCNU is fat-soluble and the membranes comprising the blood–brain barrier have little effect on the access of BCNU to cells within the brain. But this is a double-edged sword, since the fat solubility of BCNU also gives it access to normal brain cells.

In an advanced brain tumor, the blood–brain barrier can break down, so that tumor cells are exposed to chemicals in the bloodstream to a greater degree than are normal brain cells. This can be a significant advantage if the goal is to dose tumor cells with chemotherapy while sparing normal brain cells from toxicity. There have also been deliberate efforts to temporarily open the blood–brain barrier, by infusion of mannitol into the arteries supplying a tumor. Mannitol infusion can disrupt the blood–brain barrier, allowing access of water-soluble chemicals to brain cells.

Normal cells in the brain grow extremely slowly, if at all, in adulthood. Even though malignant brain tumors have a cell growth rate that can be dramatically higher than the brain in which they are growing, the growth rate of brain tumor cells is still very slow compared with many other tumors. The labeling index (LI) is a measure of the proportion of cells that take up a precursor of DNA and use that precursor to make new DNA. The LI for brain tumors increases with increasing tumor malignancy, but is generally very low: LI is about 1% in ependymoma; 2% in moderately anaplastic astrocytoma; 9% in highly anaplastic astrocytoma; and 10% in glioblastoma multiforme.¹⁰⁴ This

contrasts sharply with the LI of many other tumors. For example, ovarian carcinomas can have an LI of 18–20%, stomach carcinomas can have an LI of 27–28%, and cervical carcinomas can have an LI as high as 40%.

Both radiotherapy and chemotherapy are most effective in killing those cells that are rapidly growing. Hypoxic cells, cells that are dividing at a low rate, or cells that have entered G_0 phase of the cell cycle and are not dividing at all, are all somewhat protected from radiation. In addition, some forms of chemotherapy are less toxic to hypoxic cells, and nearly all forms are less toxic to cells that are not dividing. Even a highly malignant glioblastoma multiforme grows rather slowly, so neither radiotherapy nor chemotherapy may be effective in generating cell kill in these tumors. Therefore, some measure of resistance to therapy is conferred on a brain tumor simply because of tumor physiology. This suggests that it may be appropriate initially to treat brain tumors using chemotherapeutic agents that are selectively toxic to quiescent, nonproliferating cells. After a certain number of tumor cells have been killed and some quiescent cells have been recruited back into the cell cycle, it may be appropriate to switch agents and use a chemotherapeutic agent that is selectively toxic to cycling cells. Some experimental work with rats has shown that cell-cycle-specific agents such as 5-fluorouracil and vincristine are effective if given after BCNU. This may be because the BCNU stimulates surviving cells to divide, making them more vulnerable to toxicity from cell-cycle-specific agents.

In addition to the physiological resistance conferred by the blood–brain barrier, and by the decreased vulnerability of quiescent cells, there may also be an inherent cellular resistance to chemotherapy in some brain tumor cells. Failure of brain tumor chemotherapy is often the result of cellular resistance to the drug, achieved through a variety of mechanisms: intracellular concentrations of the chemotherapeutic drug may be low because of reduced drug uptake, increased drug efflux, or rapid intracellular binding of the drug; or resistance might occur because of rapid degradation of the drug, resistance of the target molecule to drug action, or rapid repair of drug-induced damage.¹⁰⁵ A large number of human gliomas have been brought into cell culture over the last several years, using cells removed from patients during brain tumor surgery. Typically, these cell lines display a wide range of sensitivity to common chemotherapeutic agents. There can even be heterogeneity of cell resistance among the cells of a single tumor, such that some cells of the tumor are more resistant to a given drug than are other cells of the same tumor. Eventually, cell culture studies of biopsied tumor cells may enable scientists to

screen the tumor cells of a patient against a variety of chemotherapeutic agents, to determine which agent is most successful in killing the patient's tumor cells.

Examination of histological specimens taken from human gliomas often shows large areas of necrosis within the tumor mass. It is unknown what causes tumor cells to die and necrose within the tumor, but one possibility is that these cells are inadequately supplied with metabolic substrates so that they literally starve to death. Tumor cells near the areas of necrosis usually grow at a slower rate than well-perfused tumor cells. Cells in the necrotic area, the area of slow cell growth, and the area of rapid cell growth at the tumor margin each experience quite different growth conditions. Few cells can survive near the necrotic part of the tumor, given that the availability of metabolic substrates is low and the area may be rich in toxic products released by dying tumor cells. Nevertheless, there may be some cells that survive here by becoming quiescent. If these cells do not proliferate, they become resistant to therapy, but they may be capable of reentering the cell cycle if other tumor cells are killed by therapy. Cells that are farther from the area of necrosis may also be quiescent, but they are more likely to be viable and adequately supplied with metabolic substrates. These cells may be the most likely to cause tumor recurrence if the tumor is treated with radiotherapy or chemotherapy alone. Finally, the cells at the tumor margin are well perfused and actively proliferating, so they may be fairly vulnerable to therapy. Yet these cells are also diffusely invasive into surrounding normal brain, so they are quite likely to be left behind if surgery is attempted.

Gliomas exhibit a striking degree of heterogeneity in the cellular content of DNA and in the number of chromosomes present in each cell. Studies of cells dissociated from a single tumor have shown that some cells have the normal (diploid) content of DNA, while other cells from the same tumor may be polyploid or aneuploid. Chromosomal and molecular analysis of medulloblastoma and glioblastoma multiforme show that these tumors frequently have amplified genes and may also have lost parts of certain chromosomes. Progressive gene amplification may confer a selective growth advantage on certain cells, so that over time those cells with gene amplification come to dominate the tumor cell population. Recent work on gene amplification in human medulloblastoma is consistent with this interpretation. Examination of medulloblastomas removed at surgery shows that only 3–5% of these tumors have cells with double minute (DM) chromosomes and gene amplification. Yet more than 90% of the medulloblastoma cell lines in culture have amplification or rearrangement of the *c-myc* gene. This genetic alteration appears to provide a growth advantage to medulloblastoma cell lines grown in culture, although it

may not be important to growth of the tumor in the patient.¹⁰⁶ On the other hand, 40–50% of glioblastoma multiforme have some degree of gene amplification, as shown by the appearance of DM chromosomes.

The most frequently amplified gene in human glioma codes for the epidermal growth factor (EGF) receptor.¹⁰⁶ Amplification of this gene was identified in 40% (4 of 10) of human gliomas, with the extent of amplification varying between 6- and 60-fold. In another analysis, 15 of 33 gliomas showed amplification of the EGF receptor gene. Amplification of this gene is associated with increased expression of EGF receptor on the cell surface, but it is unknown whether this has an effect on tumor cell growth. However, normal glial cells in culture respond to EGF by increasing their growth rate. Since EGF is present in most tissues, it is possible that EGF receptor expression actually stimulates tumor cell growth. Presence of EGF receptor exclusively in high-grade malignant tumors suggests that progression to malignancy may be associated with EGF receptor gene amplification. If EGF does selectively enhance glioma growth, it may eventually be possible to block tumor growth with antibodies specific to the EGF receptor protein.

Recently, evidence has been obtained that the tumor suppressor gene p53 can be lost or mutated in some brain tumors, and that loss of p53 is more common in aggressive brain tumors. Tumor tissue samples from 45 brain tumor patients were examined, to determine what proportion of the tumors carried mutations of p53, and what proportion of tumors showed evidence of other genetic mutations.¹⁰⁷ It was found that no (0 of 6) low-grade astrocytomas had p53 mutations, while an average of 31% of the more malignant tumors, such as anaplastic astrocytoma and glioblastoma multiforme, had p53 mutations. Furthermore, a certain type of mutation known as loss of heterozygosity, in which genetic information is selectively lost from a chromosome, was more common in highly aggressive tumors. Loss of heterozygosity affecting chromosome 10 was found in no low-grade astrocytomas, in 23% of anaplastic astrocytomas, and in 61% of glioblastomas. Only patients with highly aggressive glioblastoma multiforme showed both loss of heterozygosity and mutations of p53. These results suggest that mutation or loss of p53 can be involved in progression of brain tumors to a more malignant form, and that genetic mutations tend to accumulate during tumor progression.

At present, all treatment of primary malignant brain tumor is merely palliative, although significant increases in duration of survival and quality of life can be achieved in most cases. Currently, surgery is used, in some cases, for palliation of symptoms even when the tumor is considered incurable. Surgical excision of malignant glioma may be undertaken because tumor volume

reduction permits increased survival time and an opportunity to attempt adjuvant therapy. Furthermore, removal of the bulk of the tumor recruits those cells that are not actively cycling back into the cell cycle, so that they become more vulnerable to radiation or chemotherapy.¹⁰⁸ There is also an active effort by scientists to develop new therapies, and to validate experimental therapies, for brain tumor.

One of the most intriguing of the new therapies, thus far used only experimentally in rats, involves the use of a virus to infect growing glioma cells. This viral infection makes the glioma cells vulnerable to subsequent treatment with antiviral therapy.¹⁰⁹ Rats with a brain tumor were given an injection, directly into the tumor, of cultured cells that had been deliberately infected with a particular type of virus. This virus, called a retrovirus, is somewhat unusual in that it can invade dividing cells and cause these cells to produce viral proteins, but it is unable to invade cells that are not actually dividing. Since normal brain cells in adult rats do not divide, brain cells are essentially immune to the effects of the virus, while tumor cells, which are actively dividing, become infected with the virus. Viral particles are actually able to move out of their host cells and infect nearby dividing tumor cells. The infected tumor cells begin to synthesize viral proteins, which makes these cells vulnerable to an antiviral agent called ganciclovir. If rats are first injected with virally infected cells, and then are treated with ganciclovir 5 days later, the tumors often undergo complete remission and all signs of the tumor disappear.¹⁰⁹ This is an extremely clever therapeutic strategy, one that may eventually prove to be effective against human brain tumors as well, but it is important to remember that these results are only preliminary. The experiment has been done in only a few sets of rats, bearing one particular brain tumor cell line, and the experiment has not yet been replicated by other researchers. Until these findings have been extensively validated we cannot be sure that this was anything other than a fascinating experimental fluke. Nevertheless, because of the striking success with rat brain tumor, a new clinical trial has been initiated in human brain tumor patients using a very similar protocol. These new results are potentially important because they call attention to a therapeutic strategy that has been little utilized thus far: the direct transfer of specific genes into tumor cells. If tumor cells could be induced to synthesize a product toxic to themselves, or if genes that stimulate an immune response could be inserted into tumor cells, or if tumor-suppressor genes could be introduced into tumor cells, this might improve prognosis for certain brain tumor patients.

CHAPTER 13

Head and Neck Tumors

OCCURRENCE

Cancers of the head and neck are principally cancers of the mouth, the larynx, the pharynx, and the thyroid gland. The larynx is that part of the upper airway that contains the vocal cords, while the pharynx is that part of the digestive tract extending from the nasal cavity to the larynx and esophagus. The thyroid is a small gland surrounding the larynx that secretes hormones controlling growth, maturation, energy expenditure, and protein turnover rate in the body. However, the catchall category of head and neck cancer can also include cancers of the eye, the nasal cavity, the sinuses, the lip, the salivary glands, the eustachian tubes or middle ear, and the skin. The American Cancer Society has estimated that in 1990, there were 30,500 newly diagnosed patients with cancer of the oral cavity or pharynx, 12,300 new patients with cancer of the larynx, and 12,100 new patients with cancer of the thyroid. These patients, when combined, represent roughly 5% of all newly diagnosed cancer patients in 1990. Thus, even if patients with cancer at the less common sites are not included, head and neck cancers are rather common in the aggregate.

About 85% of head and neck cancers develop from the mucosa, or mucus membranes, of the upper respiratory and digestive tracts. The remaining 15% of head and neck cancers consist of cancers of skin, cancers of glandular tissue such as the thyroid or salivary glands, cancers of soft tissue (e.g., muscles, nerves, or blood vessels), and bone cancers. Squamous cell carcinoma, also called epidermoid carcinoma, is the most common form of head and neck cancer, and is derived from the layer of skin cells known as stratified squamous epithelium. Squamous cell carcinomas are usually flat and plaquelike initially, but they can grow over and replace other tissue, and they typically form a variable amount of keratin within the tumor. Keratin is a protein normally found in structures such as hair, nails, and horn, and this protein either forms a layer

on the surface of the tumor or forms structures called “keratin pearls” within the tumor mass. Overall, squamous cell carcinoma (SCC) accounts for nearly 90% of all head and neck tumors, and the following text will largely be limited to SCC of the mouth, larynx, and pharynx.

Generally, the incidence of all head and neck cancers is higher in males than in females. In the United States, white males are approximately 3-fold more likely to get head and neck cancers than white females, and black males are 2.5-fold more likely to get these cancers than black females. The incidence of laryngeal cancer specifically is 10-fold higher in men than women in the United States. In European countries of Latin origin, the discrepancy between cancer incidence in men and women is even more striking; French men are about 19-fold more likely to get cancers of the head and neck than French women.¹¹⁰ The only exception to this rule is thyroid cancer, which afflicts females 2- to 3-fold more frequently than males. The majority of head and neck tumors affect men between the ages of 50 and 60 years old.

There is a great deal of geographic variation in the incidence of head and neck cancer.¹¹¹ France has the highest European mortality from cancers of the mouth, larynx, and pharynx, with nearly 15% of all male deaths from cancer involving these sites, whereas in the United States these cancers form less than 4% of the total cancer mortality. Carcinoma of the nasopharynx represents only about 0.25% of all cancers in western Europe, but it makes up 18% of all malignancies in southern China. Laryngeal cancer incidence in France, Italy, and Brazil is more than tenfold higher than in Senegal, and blacks in the United States have an incidence of laryngeal cancer seven- to ninefold higher than blacks in Senegal.

There also appear to be racial differences in the incidence of head and neck cancer. In the United States, white males are slightly more likely (1.3-fold) to be afflicted with head and neck cancers than black males. More strikingly, women in Hawaii have a disease incidence that varies widely by race; the incidence of thyroid cancer is highest among native Hawaiian women (17.6 per 100,000), somewhat lower among Filipino (16.6 per 100,000) and Chinese women (12.1), and substantially lower among Caucasian (6.7) and Japanese women (6.2).¹¹¹

RISK FACTORS

The upper part of the respiratory and digestive tracts (mouth, larynx, and pharynx) come in contact with a wide range of potential carcinogens, including

various foods, fluids, smokes, vapors, and dusts. Any of these agents could potentially cause chronic irritation of delicate mucosal tissues, and abundant evidence has accumulated that most cancers of the head and neck result from chronic mucosal damage. Typical mucosal carcinogen exposures include alcohol or tobacco use, inhalation of wood dust, exposure to carcinogens in the nickel and chromium industry, and sun exposure. Cell damage by these carcinogens can apparently be aggravated by various vitamin deficiencies. The idea that chronic cell damage leads to cancer is supported by data showing that the incidence of second cancers is very high in cancer patients who continue to smoke and drink. Patients with oropharyngeal cancer who do not abstain have a 15–20% chance of a second primary tumor in a follow-up of only 2–5 years.¹¹²

Tobacco was one of the first carcinogens to be clearly linked to cancers of the oral cavity, because of the relatively high incidence of lip cancer among cigar and pipe smokers. Typically, lip tumors occur on the same side of the mouth on which the cigar or pipe is usually held, and the incidence of cancer on the opposite side of the mouth is much lower. Chewing tobacco also leads to an elevated risk of oral cancer, specifically in the area where the wad of tobacco is habitually held in the cheek. Tobacco carcinogens have been implicated in the causation of nearly one-third of all human cancers, so it is not surprising that tobacco is responsible for oral cancers as well. Tobacco use appears to cause a 2- to 3-fold elevation in risk of cancer at any site in the oral cavity, larynx, or pharynx.

Alcohol use also appears to cause a 2- to 3-fold elevation in risk of cancer in the oral cavity, larynx, or pharynx.¹¹³ But alcoholics have a 10-fold elevated risk of cancer at all sites compared with the general population, with the greatest increase over expected rates occurring in the head and neck, esophagus, stomach, liver, and pancreas. What is perhaps most significant, since most heavy drinkers are also smokers, is that combined tobacco and alcohol abuse appears to cause an elevation of cancer risk more than 15-fold over normal. Heavy alcohol use increases the risk of cancer at every site in the mouth and pharynx that normally comes in contact with fluid, while nearby sites that do not come in contact with fluid do not have an elevated risk. The mechanism by which alcohol increases cancer risk is unknown, since alcohol is not a carcinogen in animal tests. But alcohol is a local irritant, and it may also be a cocarcinogen, or it may act as a solvent for carcinogens, thereby improving the access of carcinogens to mucosal cells. Several different factors may interact in cancer causation in the chronic alcoholic, since the long-term alcoholic often suffers from malnutrition, immune system depression, and

chronic hormonal changes (e.g., typically, an elevation of cortisol and a depression of vasopressin and thyroxine).

Recent data implicate alcohol as a more significant risk factor than tobacco for cancers of the head and neck. Data from England and Wales show a consistent decline in mortality from cancer of the tongue during the period from 1931 to 1975.¹¹¹ The decline in head and neck cancer frequency closely paralleled a decline in the death rate from alcoholic cirrhosis. In 1931, the mortality from cancer of the tongue was nearly 8% of all cancer mortality, but mortality declined to only 1% by the end of the period in question, with no significant change in cancer survival. Over this time period, tobacco use increased, while alcohol use declined, suggesting that alcohol use is more closely linked to oral cancer incidence.

Several other carcinogens have been identified that cause an increased risk of cancer of the oral cavity, larynx, and pharynx, and most of these carcinogens are chronic irritants. For example, in India, betel (areca palm) leaf is often wrapped around a betel nut and some quicklime and chewed like gum, to obtain a stimulating, narcotic effect. This habit appears to result in an elevated risk of oral cancer. Miners of asbestos have an increased incidence of cancer of the upper respiratory and digestive tracts. Users of tobacco snuff and woodworkers exposed to wood dust both suffer an elevated risk of nasal cancer. Chronic exposure to intense sunlight is a potent risk factor for lip cancer. There is even the suspicion that chronic wounding from a poorly-fitted dental appliance can cause cancer at the wound site, although this is controversial.

The Epstein–Barr virus (EBV) has been closely linked to several different cancers of the head and neck, including Burkitt's lymphoma and undifferentiated nasopharyngeal carcinoma. Nasopharyngeal carcinoma occurs in the airway connecting the nasal cavity to the back of the mouth, where it can cause a neck mass, plugged ears, and hearing loss. The incidence of nasopharyngeal carcinoma is roughly 118-fold higher in the Canton province of China than in whites in the West, and two separate lines of evidence suggest EBV as a causative agent. Among Chinese patients with nasopharyngeal cancer, antibodies to EBV are significantly elevated with respect to a control Chinese population, and the amount of antibody in the bloodstream generally increases as the disease progresses. In fact, the presence of antibodies to EBV has even been used as a diagnostic criterion for nasopharyngeal cancer by some oncologists. Furthermore, biopsies of tumor cells regularly show expression of a characteristic viral DNA "fingerprint," suggesting that the virus is present

and active in tumor cells. However, the mechanism by which EBV induces tumor growth is unknown.

A great deal of evidence shows that there is an increased incidence of cancer in patients who are immunosuppressed to prevent transplant rejection. Patients who have received a kidney transplant and are chronically immunosuppressed are 20- to 50-fold more likely to develop cancer. The most common malignancy among transplant patients is lymphoma (relative risk elevated 35-fold), but lip cancer is the third most common tumor among these patients. Transplant patients typically suffer cancer at an earlier age than normal, with oral cancer patients as young as 8 years old reported. Tumor development is a consequence of immunosuppression, rather than any carcinogenicity of the immunosuppressant itself, since different immunosuppressants all cause the same cancers.

A number of precancerous lesions in the mouth have been described, including leukoplakia and erythroplasia.¹¹⁴ Leukoplakia usually is a small, white, clearly defined, roughened patch in the mouth, while erythroplasia is a bright red patch. Histologically, leukoplakias show hypertrophy of those cells that normally form the horny layer of skin, together with premature formation of keratin in immature cells (i.e., keratin formation is generally a sign of a mature skin cell). Nodular leukoplakias have a higher potential for malignant transformation than flat lesions, but fewer than 8% of leukoplakias prove to be malignant. Recent evidence suggests that the velvety red lesions called erythroplasia have a greater tendency to transform than do the white lesions. However, any chronic ulceration in the mouth that fails to heal within 1 to 2 weeks should be considered potentially malignant.

NATURAL HISTORY OF THE DISEASE

Often, head and neck tumors are noted early in their course by the patient, because neck tissues are not massive and a lump is easily palpated, because the tumor may be clearly visible, or because partial obstruction of an airway or the digestive tract occurs relatively early. These symptoms may be ignored by the patient because head and neck tumors are rarely painful until far advanced. Tumors of the head and neck tend to spread easily to regional lymph nodes, because the lymph drainage of the head and neck is very extensive. Thus, more than 40% of patients have one or more metastatic nodes at the time of diagnosis,

and more than 50% of tumors are diagnosed at a point when they must be considered advanced. Therefore, any head and neck cancer symptom of more than 2 weeks' duration should be evaluated by a physician.

Signs and symptoms of head and neck cancer are highly variable, depending on which tissue is involved.¹¹⁵ Generally, one of the most common symptoms at presentation is an expanding, painless mass at some location in the neck, which may or may not involve a lymph node. Such a growing mass may be found in the salivary gland or thyroid, as well as tissues of the oral cavity, larynx, and pharynx. A persistent, nonhealing sore or ulcer on the lip, or in the mouth, may be cancerous, particularly if the edge of the ulcer is raised, hardened, and has a "rolled-under" appearance. Usually, these oral lesions are not particularly painful, and they often arise in white patches of leukoplakia. Repeated episodes of acute sinus pain can be indicative of a sinus tumor, especially if there are numerous unexplained nosebleeds (epistaxis). A persistent sore throat is commonly noted in cancer of the pharynx, particularly if cancer involves one or both tonsils. Cancer of the base of the tongue (Fig. 42) is usually associated with speech problems and ear pain, although ear pain can also be indicative of cancers of the nasopharynx and pharynx. Hoarseness is a common symptom of laryngeal cancer, particularly if cancer involves the vocal cords. Finally, a less common symptom of head and neck cancer is a fever "of unknown origin," caused not by microbial infection but by the tumor itself through an unknown mechanism.

Poorly differentiated (anaplastic) tumors tend to have a short doubling time, to contain cells with an aneuploid number of chromosomes, and to metastasize sooner than well-differentiated tumors. Well-differentiated tumors often arise from epithelial cells of the larynx and mouth, including the lips, the hard and soft palate, and the base of the tongue. Moderately differentiated tumors occur in the sinuses and on the floor of the mouth. Poorly differentiated or undifferentiated tumors tend to occur in the nasopharynx, the tonsil, the mobile portion of the tongue, and the lower part of the pharynx. Tumor invasion tends to occur along rather stereotyped pathways: if the tumor is underlain by muscle, it extends along the muscle fibers; if the tumor is underlain by bone or cartilage, it extends along the path of least resistance; but if the tumor is underlain by glandular or lymphoid tissue, then tumor spread is less predictable and more widespread.

The generalization that large tumors have a poorer prognosis is generally true for cancers of the head and neck. However, SCC of the oral cavity tends to be more extensive at the tissue surface than in deeper tissue layers and, if such

BIOLOGY SPECIFIC TO THE TUMOR

An unusual feature of most tumors of the head and neck, relative to tumors at other sites, is that usually the blood supply to the tumor is entirely dependent on a single artery, the external carotid, and its branches. This can be a significant advantage because it should be possible to treat the tumor with intra-arterial chemotherapy. Infusion of a chemotherapeutic agent directly into the artery perfusing the tumor means that the tumor will be subjected to extremely high levels of drug, while the rest of the body will experience the drug only after it has been diluted into a larger blood volume. Although the lymphatic drainage of the head and neck is extensive, it is also relatively well isolated from surrounding tissues. Thus, while a head and neck cancer may seed itself regionally, most such cancers do not metastasize systemically until quite late in their course.

Multiple cancers of the head and neck are rather common, being found in 7–9% of patients at diagnosis.¹¹⁰ Nearly 20% of patients with cancers of the head and neck either have, or later develop, what appears to be a second primary tumor. Common sites for these second malignancies include other head and neck sites, the lung, or the esophagus. Three years after initial treatment, the patient actually has a higher probability of developing a second primary tumor than of having the first primary tumor recur at its original site.¹¹⁷ The development of multiple primary tumors may be a function of the fact that irritants such as alcohol or tobacco smoke tend to affect all tissues of an area equally, so there may be ample opportunity for formation of a second primary cancer. Alternatively, the appearance of multiple primaries in the head and neck may be a consequence of the fact that lymph drainage is extensive, so that the lymphatics provide an easy thoroughfare by which cancer cells disseminate. If a metastasis grew very slowly, a secondary (metastatic) tumor could easily be mistaken for a second primary tumor. A final possibility is that, in the course of surgery to remove the primary tumor, cancerous cells may be dislodged and may disseminate through the surgical field. Cytologic examination of washings obtained from surgical wounds of cancer patients are positive for the presence of cancer cells in 10–25% of cases.¹¹⁶

Head and neck tumors generally tend to remain as local or regional disease for a fairly long period of time. Autopsy studies have shown that 30–50% of head and neck cancers metastasize systemically to form clinically silent tumors, but fewer than 10–15% of such tumors form metastases that are symptomatic. This situation is really quite different from most other types of

carcinoma, where metastatic disease is very likely to be the cause of death. For this reason, an aggressive therapeutic response to head and neck cancer is warranted, and surgery is considered to be the treatment of choice. Radiation therapy has also found a large role in treatment of these cancers, since radiation is very good at controlling local recurrence, although it provides no protection from systemic metastasis. Distant metastases are typically preceded by local recurrence, and only 10–20% of patients develop distant metastases without first experiencing local recurrence. When head and neck cancers do metastasize, the most common sites are the lungs, liver, and bones, with bones such as the spine, the ribs, and the skull especially vulnerable.

Lymphatic involvement is the most important prognostic factor for cancers of the head and neck. The presence of even a single positive lymph node decreases the likelihood of survival by as much as 50%. The various degrees of lymphatic involvement are defined by the presence or extent of lymph node invasion; the site of the involved lymph node; rupture of the lymph node capsule; and the number of tumor cells trapped in lymph vessels.

Head and neck cancers can develop as a consequence of immune system deficiencies. The increased incidence of cancer in older persons may well be a consequence of decreased immune system function, since with increasing age there is also a decrease in antibody production and a decrease in the strength of the immune response to certain antigens. An investigation of immune system competence in patients with head and neck cancer has shown that these patients often show anergy, which is the total absence of a measurable response to a challenge antigen. Anergy may develop in cancer patients during the course of their disease, as when a carcinoma *in situ* becomes invasive, or when a tumor begins to metastasize.

Recent research has shown that the primary site of EBV infection is probably epithelial cells of the nose and mouth. Once infected with EBV, the person harbors the virus for his or her entire lifetime, although the tissue reservoir of the virus is unknown. An increased level of antibodies against EBV is often present months to years before nasopharyngeal cancer develops, and EBV antibodies actually define a population in south China at high risk for nasopharyngeal cancer. Thus, EBV antibodies actually herald the development of cancer, suggesting that there may be a relationship between antibody production and viral carcinogenesis. Recently, scientists attempted to define the events associated with viral carcinogenesis using the cultured human epithelial cell line HT-29. Normally, HT-29 cells can resist invasion by EBV, but when the virus was presented to these cells together with an antibody directed against the

virus, the virus–antibody complex was taken up by HT-29 cells. Thus, expression of an antibody to EBV actually increased the vulnerability of an epithelial cell line to viral infection. This may explain why EBV induces carcinogenesis in cells that are normally able to exclude virus.¹¹⁸

Recent evidence suggests that mutations of the p53 tumor suppressor gene are important in the development of head and neck cancer.¹¹⁹ An analysis of 30 human tumor samples or cell lines derived from patients with SCC of the head and neck (Stages I–IV), revealed that 53% of these tumors showed mutations of p53. Patients with p53 mutations tended to have a shorter disease-free interval before tumor recurrence, although no relationship was observed between tumor stage and p53 mutation. The presence of p53 mutation appears to identify a population of patients with aggressive tumors who are at greater risk of tumor recurrence.

CHAPTER 14

Lung Cancer

OCCURRENCE

Lung cancer is now the leading cause of cancer death in both men and women. Cancers of the lung and respiratory tract account for about 35% of all cancer deaths in men and about 19% of cancer deaths in women. The American Cancer Society has estimated that primary carcinoma of the lung affected more than 157,000 men and women in the United States in 1990, and many of these patients died within a year of diagnosis. The death rate for lung cancer in 1980 was roughly 17 times higher than in 1930 for men, and 10 times higher than in 1930 for women. In the past decade, the death rate for women has been increasing more rapidly than for men, probably because of the relatively recent increase in the incidence of smoking in women. Cancers of the lung and bronchus account for 88% of all cancers of the respiratory tract, with the remainder being cancers of the larynx (9%) and the nasal cavities (3%).

Four principal types of lung cancer have been recognized.¹²⁰ The most common type is squamous cell (or epidermoid) carcinoma, which accounts for about 33% of all lung cancers. This type of cancer has recently undergone a striking decline in incidence (or at least diagnosis); 20 years ago about 49% of all lung cancers were classified as squamous cell carcinoma. The next most prevalent type of lung cancer is small-cell lung carcinoma (SCLC), also called "oat cell" carcinoma, which accounts for 25% of lung cancer diagnoses. Adenocarcinoma constitutes another 25% of new lung cancer cases, while most of the remaining new cases (16%) are large-cell undifferentiated carcinomas. The two histologic types most closely associated with smoking, squamous cell carcinoma and SCLC, are more prevalent in men, while adenocarcinoma accounts for a larger proportion of cases in women.

RISK FACTORS

By far the most important risk factor for development of lung cancer is tobacco use, whether in cigarettes, cigars, or pipes.¹²¹ A Congressional study has reported that health costs from the adverse effects of smoking account for more than \$100 billion a year in medical bills and lost worker productivity. Tobacco smoke is associated with increased incidence of cancers of the oral cavity, esophagus, larynx, kidney, bladder, and pancreas, as well as cancer of the lung. Tobacco-related malignancies account for about one-third of all cancer deaths among men in the United States, and for 5 to 10% of cancer deaths among women in the United States. It is particularly ironic that the United States government subsidizes tobacco growers to produce tobacco, then often must subsidize the medical costs of those who contract cancer because of tobacco use.

Ninety percent of all lung cancer patients are smokers, and the rare nonsmoker who contracts lung cancer is often suspected to have a primary tumor located elsewhere that has metastasized to the lung. Tobacco smoke contains several potent carcinogens, all of which are capable of inducing malignant transformation in animal experiments. There is a close relationship between lung cancer death rate and total exposure to tobacco smoke. The risk of contracting lung cancer is 60- to 70-fold higher for a man smoking two packs a day for 20 years than for a nonsmoking man. Although the chance of contracting lung cancer decreases following smoking cessation, relative risk may never return to the level of risk for the nonsmoker.

The recent increase in incidence of lung cancer among women is almost certainly related to the relatively recent increase in smoking by women. Women who smoke have a 35-fold higher risk of contracting any of several different types of lung cancer, although it was also found that a family history of lung cancer increased the risk of disease by 2-fold.¹²² Data on smoking prevalence and lung cancer incidence, collected for men and women in England and Wales since 1900, show the relationship between smoking and cancer. Smoking by men began to increase before the turn of the century, but lung cancer incidence did not undergo a striking increase until about 1930. The incidence of smoking by women increased in about 1940, but lung cancer incidence did not undergo a corresponding increase until about 1960. These data show that there is often a long lag time between exposure to a carcinogen and development of the associated cancer. The lag between carcinogen exposure and development of

cancer probably also accounts for why age is a risk factor for lung cancer; the highest incidence of lung cancer is in persons between 55 and 65 years of age.

A great deal of recent controversy has surrounded the subject of "secondhand smoke." The incidence of lung cancer appears to be two- to three-fold higher in nonsmoking long-term cohabitants of smokers than in a nonsmoking population not exposed to cigarette smoke on a daily basis. However, it is unclear whether short-term contact with tobacco smoke increases lung cancer risk. In general, incidence of cancer in nonsmokers exposed to cigarette smoke is far lower than that in smokers.

While 90% of lung cancer patients are smokers, only about 1 in 10 smokers actually develop lung cancer. This suggests that there are other factors that affect the risk of smokers contracting lung cancer. There is evidence that cigarette smoke can interact with a number of other carcinogens present in the environment. Therefore, the lung cancer risk of smokers may be elevated according to their exposure to tobacco cocarcinogens. Alternatively, certain smokers may simply be inherently more resistant to the carcinogenic effects of smoking.

There has been a recent surge of concern about exposure to radon in the home, because radon is emitted from the soil in many parts of the United States. Radon is a radioactive gas that is odorless and tasteless, and can be taken into the lung during normal breathing. It is the single most important source of radiation exposure for the general public, accounting for about two-thirds of the natural (i.e., nonmedical) radiation exposure of most people.¹²³ Radon was first identified as a lung carcinogen from epidemiological studies of miners exposed to high levels of radon, yet the total radon doses experienced by these miners is poorly known. The average duration of radon exposure in these miners was 500 months, and only miners who were exposed to fairly high levels of the gas showed an increased risk of lung cancer. Therefore, it is uncertain to what extent risk attaches to the low-level radon exposure commonly experienced by the general public. Even though it is not yet possible to estimate what level of risk is caused by radon exposure in the home, some laboratory studies suggest that radon could be a significant lung carcinogen. It appears to be an especially powerful cocarcinogen when combined with tobacco smoke, so further study of the radon problem is needed.¹²⁴

Environmental or occupational risk factors associated with increased incidence of lung cancer are varied. Lung cancer is somewhat unusual in that a relatively large number of carcinogens are known to interact with each other as

cocarcinogens, and to increase the risk of lung cancer. For example, exposure to asbestos appears to have a major synergistic interaction with tobacco use in the development of lung cancer. Cocarcinogens for lung cancer may include arsenic, asbestos, diesel exhaust, chromium compounds, coal by-products, and radon. Manufacture of chemicals and nuclear weapons, or coal, uranium, and iron mining all can lead to significant occupational exposure to various cocarcinogens for lung cancer. No conclusive evidence yet exists linking exposure to air pollution with development of lung cancer. Studies that correlate the incidence of lung cancer with increased levels of pollutants in urban air are complicated by the fact that most lung cancer victims are smokers, whose exposure to carcinogens occurs predominantly through cigarette smoke.

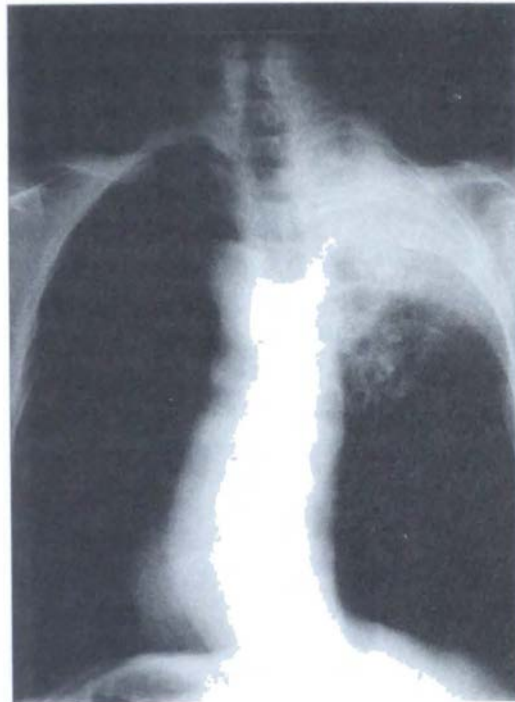
Adenocarcinomas occur more frequently in areas of the lung scarred by chronic inflammatory changes. Any disease that induces pulmonary fibrosis, including chronic interstitial fibrosis and bacterial or fungal infection, can be associated with later development of lung cancer. There may also be familial or genetic factors involved in the development of lung cancer, since certain families tend to be prone to development of lung fibrosis. However, all of these additional risk factors pale to insignificance when compared with the risk conferred by tobacco usage.

NATURAL HISTORY OF THE DISEASE

The lung can be visualized as an inverted tree, in which the leaves of the tree are the air sacs, or alveoli, of the lungs. The branches of the tree are the bronchial tubes, which bring air to and from the alveoli. The trunk of the tree is the main bronchial tube, which originates in the trachea, or windpipe. Lung cancer typically begins with microscopic changes in the epithelial cells lining the bronchial tubes. Cellular atypia often progresses through carcinoma *in situ* (noninvasive carcinoma) to invasive carcinoma before the disease causes significant symptoms. Because the affected cells are those that line the airways of the lung, malignant growth of these cells ultimately obstructs the airways. But the lung generally has a larger capacity than is used by a sedentary patient, so obstructive loss of lung capacity is often not noticed by the patient at first.

Only about 5 to 15% of lung cancer patients are detected while still asymptomatic, during the course of a routine chest X ray. Most lung cancer patients are diagnosed because they present to the physician with certain typical clinical signs or symptoms (Fig. 43). The most common symptoms associated

Figure 43. Chest X ray of a 70-year-old male patient who had smoked cigarettes for over 50 years. He presented with a complaint of "chronic bronchitis" and reported episodes of coughing with production of bloody sputum. A chest X ray taken earlier had noted a lesion at the apex of one lung that appeared to be an infection, but laboratory tests for tuberculosis had been negative. The chest X ray shown here was taken one year after the first film, and shows that the lesion (top right) had progressed and now resembled a typical lung tumor. Note the clear appearance of the lung to the left, in sharp contrast with the clouded appearance of the involved lung; there is evidence of infection and scarring as well as tumor in this lung. Lung examination and biopsy confirmed that the tumor was a well-differentiated squamous carcinoma. (Photograph courtesy of Dr. Stephen Marglin and Dr. James A. Nelson, University of Washington School of Medicine.)



with lung cancer include a persistent cough, a cough producing blood, wheezing or high-pitched sighing sounds during breathing (indicating respiratory obstruction), shortness of breath, pain in the chest wall, or inflammation of the lung with fever and a productive cough. While some of these symptoms may seem trivial, all can be indicative of serious illness, particularly when symptoms persist for more than a few days.

Signs and symptoms associated with regional spread of the tumor can include obstruction of the trachea, compression of the esophagus with difficulty in swallowing, recurrent paralysis of the larynx with hoarseness, and Pancoast's or Horner's syndrome. Pancoast's syndrome is a concurrence of unusual symptoms including pain and tingling of the arm and hand, constriction of the pupil of the eye, and paralysis of the muscle that raises the eyelid. This aggregate of symptoms indicates that a lung tumor is pressing on a nerve plexus near the apex of the lung. In addition, this syndrome may involve partial destruction of the first and second ribs, adjacent to the involved nerve plexus. Horner's syndrome is another odd collection of symptoms including drooping of the eyelid, contraction of the pupil of the eye, recession of the eyeball within

the socket, and impaired facial sweating. This syndrome indicates that a lung tumor has invaded the sympathetic nerves of the eye. Often Pancoast's and Horner's syndromes coexist in the same patient. All of these signs and symptoms are cause for real concern, because they suggest that the primary tumor has begun to metastasize.

There are additional signs and symptoms associated with broad regional spread of lung cancer. These symptoms include: extension of tumor into the pericardial sac around the heart with resultant heart compression, arrhythmia, or cardiac failure; obstruction of lymph return to the bloodstream with resultant accumulation of fluid in the lungs (pleural effusion); extensive involvement of the lung, with shortness of breath and low oxygen content of the blood; and superior vena cava syndrome. Superior vena cava syndrome is characterized by swelling, water retention, or blood engorgement of the face, neck, and arms, a nonproductive cough, shortness of breath, and, occasionally, bluish star-shaped markings overlying large veins. Vena caval syndrome occurs because the tumor is pressing on and partially occluding the superior vena cava, the large vein that returns blood from the head, neck, and arms to the heart.

Lung cancer often induces extremely varied effects at sites remote from the tumor.¹²⁰ These remote effects may be the first symptoms noticed by the patient, and they are easily mistaken for metastatic disease. These symptoms form syndromes that are often called paraneoplastic syndromes, to indicate that they are associated with cancer. In most cases the means by which the tumor induces a paraneoplastic syndrome is unknown, although some syndromes arise because lung tumors secrete hormones in an inappropriate manner. Often a paraneoplastic syndrome is relieved by successful treatment of the primary tumor.

Paraneoplastic syndromes with a systemic (widespread) effect are present in about 12% of lung cancer patients (Fig. 44). Squamous cell carcinomas of the lung can secrete ectopic parathyroid hormone, causing elevated blood levels of calcium (hypercalcemia) and depressed levels of phosphate (hypophosphatemia). SCLCs can secrete ectopic antidiuretic hormone (ADH), resulting in abnormally low levels of sodium ions in the blood (hyponatremia), which can cause a confused mental state, lethargy, and seizures. SCLCs can also secrete ectopic adrenocorticotrophic hormone (ACTH), causing Cushing's syndrome. This syndrome involves obesity of the trunk, facial swelling, acne, hypertension, carbohydrate intolerance, elevated protein metabolism, and psychiatric disturbances, and can also cause cessation of the menstrual period (amenorrhea) and increased body hair (hirsutism) in women. Paraneoplastic syndromes

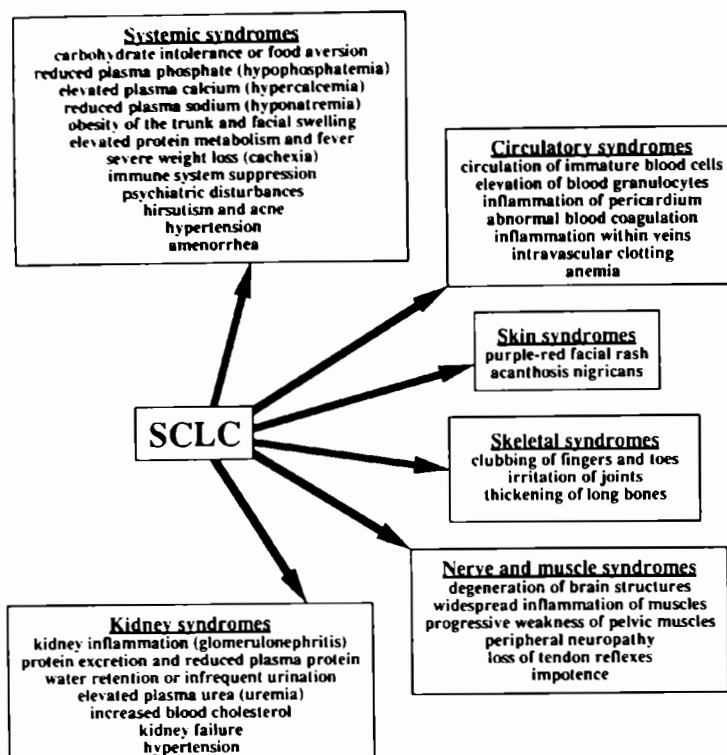


Figure 44. Summary diagram of various paraneoplastic syndromes associated with small-cell lung carcinoma (SCLC). While the mechanisms causing these syndromes are largely unknown, evidence is accumulating that ectopic hormone production by a tumor may cause many of these syndromes.

associated with lung cancer can also include systemic symptoms of unknown origin, such as: loss of appetite and food aversion (anorexia), poor nutritional state, general wasting (cachexia), and severe weight loss (seen in about 30% of patients); fever (20% of patients); and suppression of the immune system with consequent vulnerability to infection. The skeletal or connective tissue of the body is affected by paraneoplastic syndromes associated with lung cancer in about 30% of lung cancer patients. Another 8% of patients suffer paraneoplastic syndromes of the blood or circulatory system. A small number (1%) of lung cancer patients experience dramatic paraneoplastic syndromes that affect both muscles and nerves, while another 1% of patients have paraneoplastic syndromes that affect either the skin or the kidneys.

These very diverse paraneoplastic syndromes can manifest themselves before there is a clear indication of a tumor in the lung. This is particularly true for a heavy smoker, in whom lung function may be so impaired by smoking that the extra impairment of a tumor is not evident until the tumor is quite large. This can make diagnosis of lung cancer very difficult in some patients.

Non-SCLC lung cancers (squamous cell carcinoma, adenocarcinoma, and undifferentiated large-cell carcinoma) are generally considered as a group quite distinct from SCLC. The reasons for this dichotomy between SCLC and non-SCLC are twofold. First, histology is not an important predictor of survival among patients with different types of non-SCLC, so it seems legitimate to treat these cancers as a group. Second, lung cancer staging predicts survival well for non-SCLC lung cancers, but staging is a very poor predictor of survival for SCLC patients.

The specific pathology of a lung cancer can only be definitively known if a biopsy sample is examined by a pathologist. Although distinction of SCLC from non-SCLC can be difficult using only cells rinsed or scraped off the walls of the bronchi, the histological appearance of an excised tumor is usually distinctive. Identification of a particular histologic type is useful in determining patient prognosis, but within a particular type, tumor grade (i.e., the degree of anaplasia) is not a good predictor of prognosis.

Lung cancer staging is important to help determine treatment modality and patient prognosis, and for the accurate evaluation of treatment results. Typically, lung cancer staging consists of two parts. The first part, called anatomic staging, is a determination of the precise location and extent of the tumor. The second equally critical part, called physiologic staging, is an assessment of the general health of the patient and the patient's ability to withstand therapy directed against the tumor. Physiologic staging is important because many long-term smokers suffer from chronic bronchitis, chronic obstructive pulmonary disease, or heart disease in addition to lung cancer. Thus, it is critical to determine whether the tumor can be successfully resected with a standard surgical procedure, but it is equally critical to determine whether the health of the patient is sufficiently good that the patient can tolerate surgery.

Physiologic staging is also important because the general health of the patient may be compromised by the metastatic ability of lung cancer. Studies have shown that lung cancer can metastasize to virtually every organ system of the body. Common clinical problems related to metastatic lung cancer can include: brain metastases with neurologic deficits; bone metastases with pain

and pathologic fractures; bone marrow invasion with reductions in various circulating components of the blood; liver metastases causing liver dysfunction, anorexia, biliary obstruction, and pain; lymph node metastases that can be painful and may be associated with immune system depression; and metastases to the spine with cord compression and paralysis.

In most hospitals, SCLC is simply described as being either limited or extensive at diagnosis. A more exhaustive effort at staging SCLC is usually not undertaken, since survival is not predicted with any more accuracy by a thorough staging. Limited stage disease indicates that the tumor is confined to only one side of the chest, although regional lymph nodes on that side may be involved. Generally, the term limited stage is reserved for tumors that can be encompassed within a tolerable radiation port. This stage is found in only about 40% of all SCLC patients, while any other manifestations of SCLC belong to the extensive stage disease category. Accurate evaluation of extensive disease usually requires brain, liver, and bone scans, as well as bone marrow biopsy. Even limited SCLC should be considered a systemic disease at diagnosis because of the propensity of SCLC to metastasize widely by the time it is diagnosed.

BIOLOGY SPECIFIC TO THE TUMOR

Some encouraging progress has been made recently in our understanding of the biology of lung cancer. Researchers at the National Cancer Institute have been able to culture more than 100 different cell lines derived from primary lung tumors excised at surgery. The behavior of these tumor cell lines has been studied in culture for some years now, resulting in a better understanding of the growth requirements, biochemistry, molecular biology, and drug resistance of this diverse group of tumors. It may seem a rather trivial thing to be able to grow tumor cells in culture, but this is actually a major accomplishment. Only very recently has it become possible to grow human lung cancer and breast cancer cell lines in culture, so it has been difficult to identify drugs that are effective against these cancers.

An important development was the determination of a cell medium in which SCLC cells could grow well.¹²⁵ Often cells are grown in a nutrient medium supplemented with blood serum derived from fetal calves. While this medium may permit rapid growth of cells in culture, fetal calf serum is an undefined broth, potentially containing both growth factors and growth inhibi-

tors, as well as other unidentified proteins and nutrients. A serum-free growth factor-supplemented medium has now been developed for SCLC cells, so that these cells can be grown in a completely defined medium. This offers several important advantages: growth of SCLC cells is selectively favored, and non-neoplastic cells from the tumor stroma are weeded out over time; growth-inhibiting factors can be eliminated from the medium; the effect of various growth factors or chemotherapeutic agents on cell growth can be tested; and products synthesized and released by the SCLC cells can be easily identified. Interestingly, the defined medium contains several unexpected ingredients. The hormones hydrocortisone, insulin, and estradiol are present, as is transferrin, a protein that is involved in the transport of iron into cells, and selenium, a metallic element chemically similar to sulfur. It is unknown why this odd collection of ingredients is needed to permit rapid growth of SCLC cells, but it is reasonable to expect that a tumor growing in a patient might have similar requirements for cell growth.

Non-SCLC cell lines have also been grown in cell culture, but typically these cells are more difficult to grow and have more stringent growth requirements. These cell lines tend to grow with an epithelial (skinlike) morphology, and squamous cell carcinoma cell lines will often form glandlike, differentiated structures as they grow. The growth rate of non-SCLC cells in culture is roughly comparable to that of SCLC cells in culture.

On the basis of work with cultured SCLC cells, a clinically significant feature of this cancer has been elucidated.¹²⁶ SCLC cells have been divided into two types: “classic” SCLC cells, which grow relatively slowly, tend to be sensitive to both radiation and chemotherapy, and generally do not show amplification of any oncogenes; and “variant” SCLC cells, which tend to grow more rapidly, are resistant to both radiation and chemotherapy, and show amplification of the *myc* family of proto-oncogenes. Classic SCLC cells are typically derived from newly diagnosed patients who have not yet been exposed to therapy. Variant SCLC cells are more likely to be derived from patients suffering a relapse after previous treatment for SCLC. Patients with classic SCLC tumors often show a marked regression of the tumor following therapy, but these patients often relapse with tumors of the variant type, which have a poor response to therapy.

One particular oncogene, *c-myc*, is frequently amplified in SCLC variant cell lines. If a slow-growing classic SCLC cell line is transfected with the *c-myc* oncogene, that cell line is transformed to have variant characteristics. The *c-myc* gene is also frequently amplified in biopsy specimens from patients with

variant SCLC tumors. Thus, the *c-myc* gene product may be important in the establishment, maintenance, or malignant behavior of variant SCLC. Another oncogene, called *ras*^k, is frequently amplified in non-SCLC tumor cells, particularly in adenocarcinoma of the lung. Amplification of this gene is associated with enhanced expression of a protein called p21, which can be immunologically detected in about 15% of non-SCLC tumors. Altered expression or altered function of p21 is apparently sufficient to cause malignant transformation in many cells.

Recent research has identified the p53 tumor suppressor gene as a gene that is frequently lost or mutated in many different cancers, including cancers of the lung. The protein coded for by the p53 gene is a DNA-binding protein, which appears to control the rate of DNA transcription and may also control the progress of the cell through the cell cycle. When the p53 gene is lost or mutated, the tumor suppressor function of the gene is also lost. However, if tumor cells are transfected with a functional p53 gene, cell transformation is suppressed.¹²⁷ The DNA isolated from a range of human lung tumors was sequenced, to determine the prevalence of p53 mutation in lung tumors, and to determine the degree of correspondence between p53 expression and tumor malignancy.¹²⁸ It was found that none of the normal tissue samples had mutations of p53, but 26% (7 of 27) of lung adenocarcinomas, 50% (3 of 6) of large-cell undifferentiated carcinomas, and 55% (12 of 22) of squamous cell carcinomas had detectable mutations of p53. In the highly malignant SCLC, the rate of detectable p53 mutation was 74% (20 of 27). Thus, p53 mutation is exclusively found in malignant cells, and mutation is more prevalent in highly malignant lung tumors. More than half of the p53 mutations detected were of a type consistent with damage to DNA by tobacco smoke.

Work with cultured cells has also led to a fuller appreciation of the variety and potential importance of proteins synthesized and released by SCLC cells. SCLC tumors are thought to be derived from cells whose original function was to synthesize and secrete certain hormones. It is often the case that SCLC cells, both in culture and in a tumor, secrete a wide variety of hormones and other bioactive proteins. These molecules may be important to the continued growth of the tumor cells, and can potentially have powerful effects on a patient. For example, a small protein called bombesin, or gastrin-releasing peptide, is synthesized by almost all SCLC cells in culture.¹²⁹ When SCLC cells are grown in culture, adding bombesin to the medium stimulates SCLC cells to grow more rapidly. If an antibody to bombesin is added to cultured SCLC cells, so that the antibody binds all of the free bombesin, growth rate of the tumor cells is

suppressed (Fig. 24). Apparently, SCLC cells synthesize and release bombesin, which then stimulates further growth of SCLC cells, forming a self-stimulatory loop. However, high levels of bombesin are seldom found in blood serum of patients with SCLC, either because SCLC cells in a tumor do not synthesize the protein or because the protein is rapidly metabolized by the patient.

Numerous other protein hormones are synthesized and released by SCLC cells grown in culture. Many of these hormones are also found in blood serum taken from SCLC patients, suggesting that the tumors secrete ectopic hormones. The ectopic hormones most frequently found in SCLC patients include ACTH, β -endorphin, oxytocin, vasopressin, gastrin, glucagon, insulin, and calcitonin. In fact, calcitonin may be able to serve as a tumor marker, because the amount of calcitonin in blood serum appears to reflect tumor burden and may be able to serve as an indicator of tumor response to treatment. In addition, a protein called neuron-specific enolase (NSE) is elevated in the blood serum of 70% of patients with SCLC. Serial NSE measurements following treatment closely reflect clinical response to therapy, and rising levels of NSE precede clinical evidence of relapse or progressive disease. Another protein, called creatine kinase (CK-BB), appears to reflect the extent of progression of SCLC: 41% of patients with extensive stage disease had elevated serum levels of CK-BB, while only 2% of patients with limited stage SCLC showed elevated levels of this protein. Serial measurement of serum levels of CK-BB shows that this protein is an excellent correlate of clinical response to therapy.¹³⁰

Although ectopic hormone production may cause the paraneoplastic syndromes that so frequently accompany SCLC, many of the ectopic hormones found in patients appear to be biologically inactive. There is often a discrepancy between the quantity of hormone detected in patient serum and the severity of the paraneoplastic syndrome. However, this discrepancy between hormone dose and systemic response could arise through secretion of a random collection of active hormones by the tumor, some of whose effects are canceled out by other hormones.

Non-SCLC cells are distinctly different from SCLC cells in culture. Non-SCLC cells have been reported to release fewer hormones in culture and, perhaps coincidentally, non-SCLC tumors are less often associated with paraneoplastic syndromes. However, cultured non-SCLC cell lines do release ACTH, vasopressin, and calcitonin. Most epithelial cells have receptors for a growth factor called epidermal growth factor (EGF), and cultured non-SCLC cells are no exception to this rule. Biopsy specimens of squamous cell lung cancer express very high levels of the EGF receptor, while adenocarcinoma and

large-cell carcinoma specimens express lower levels. In contrast, biopsy specimens of SCLC express little or no EGF receptor activity.

A great deal of work with pathological specimens of both SCLC and non-SCLC tumors has led to an appreciation of the relationship between these tumor types. While SCLC and non-SCLC cell lines are distinct from each other in culture, these distinctions often blur in an individual tumor. Many adenocarcinomas and large-cell carcinomas appear to be composed of cells that, on closer examination, physically resemble cells from an SCLC tumor. Certain non-SCLC tumors can be shown to share biochemical similarities with SCLC tumors. Probably both SCLC and non-SCLC tumors are derived from the same or similar types of cells, so it should not be too surprising that the clear dichotomies we draw for the sake of simplicity often break down in the face of reality.

CHAPTER 15

Breast Cancer

OCCURRENCE

Breast cancer is currently the second leading cause of cancer death in women, and about 26% of all cancers in women are cancers of the breast. The American Cancer Society estimated that in 1990, nearly 150,000 women and over 1000 men were diagnosed with breast cancer in the United States. Ductal adenocarcinomas are the most common cancer of the breast, with medullary carcinomas the second most prevalent breast cancer.

Since the early 1950s, the 5-year survival rate for all stages of the disease has improved from 60% to 75% in white women, and from 40% to 63% in black women. However, these figures may be somewhat misleading. In the 1950s, the average lesion was 3–4 cm at diagnosis, and 65% of patients had lymph node involvement. In the 1980s, the average lesion was 2 cm at diagnosis and only 33% of patients had positive lymph nodes. Fewer patients now have distant metastases at the time of diagnosis, suggesting that the increase in survival rate of breast cancer patients is largely attributable to public health efforts to educate women about the benefits of regular self-examination of the breast. About 90% of breast masses are found first by the patient, either accidentally or during a routine self-examination. The remaining 10% of breast masses are discovered by a physician or by mass screening techniques such as mammography.

There has been considerable success in the effort to incorporate mammography into the routine physical examination of women over about 50 years of age. A large-scale study by the Health Insurance Plan of New York evaluated mammography as a screening tool in 62,000 patients. A 10-year follow-up of this group of women showed a 30% reduction in mortality for women 50 or older who had been screened, compared with an unscreened control group. Since screening large numbers of asymptomatic women by mammography

requires exposing these women to ionizing radiation, the risks and benefits of mammography have been hotly debated. Radiation is a potent carcinogen for breast tissue, as shown by the increased incidence of breast cancer among Japanese atom bomb survivors. But atom bomb survivors typically received large doses of radiation (0.10 to > 5.00 Gray) to the breast, whereas high-quality mammograms can be obtained with a very low radiation dose level (< 0.01 Gray). In general, the benefits of mammography appear to greatly exceed the risks.

RISK FACTORS

The concept that certain risk factors put a person at greater risk for developing a particular cancer has generally been very useful in oncology. However, this concept seems inadequate for breast cancer, since 1 of every 11 women with no known risk factors will develop the disease anyway. Nevertheless, a great deal of effort has been spent defining risk factors for breast cancer because the disease is so common.

The greatest risk factor for breast cancer is obviously being female, since women are more than 100 times more likely to get breast cancer than men. There is some suggestion that men who have two X chromosomes (i.e., XXY males) are at greater risk for contracting breast cancer, but there has been little study of this issue because the disease is so uncommon in men. But it is known that men with altered estrogen metabolism, gynecomastia (male breasts), testicular damage, or testicular atrophy (from mumps) are at greater risk than most men of developing breast cancer.

Among women, the greatest risk factor for breast cancer is age. Breast cancer incidence in women in the United States and in Denmark roughly doubles between age 35 and age 40. Breast cancer incidence in the United States continues to rise thereafter, with women 80 years of age about four times as likely to get breast cancer as women 40 years old. However, in certain low-incidence countries, the incidence of breast cancer plateaus or even declines after age 50, so that there is little or no increasing risk with increasing age. The existence of a plateau at about age 50 suggests that premenopausal breast cancer may actually be a somewhat different disease than postmenopausal breast cancer. However, this plateau could also indicate that there are different causative factors before and after menopause, or that menopause itself exerts an effect on the progression of established disease.

Women who have a first-degree relative (e.g., grandmother, mother, sister, aunt, or daughter) with breast cancer are at an increased risk of contracting breast cancer themselves. A woman's risk of developing breast cancer is increased 1.5- to 3-fold if one first-degree relative had breast cancer, and 5- to 10-fold if more than one first-degree relative had breast cancer. Risk can be inherited from either the maternal or the paternal line, and risk is greatest when one or more immediate family members developed premenopausal breast cancer. In familial breast cancer, there is also a greater likelihood of developing bilateral breast cancer (cancer affecting both breasts). Women whose mothers had bilateral breast cancer prior to menopause have a breast cancer risk that is 9-fold higher than normal; over 50% of these women will eventually contract breast cancer.¹³¹ Women with a past history of benign breast disease are also at a higher risk of contracting breast cancer, particularly if there was epithelial hyperplasia with cellular atypia.

Strong differences exist from one country to another in the incidence of, and mortality from, breast cancer. Japanese women have a nearly 5-fold lower incidence of breast cancer than women in England and Wales, and more than a 3-fold lower incidence than American women. This suggests that genetic differences between the races may be involved in breast cancer incidence. However, this idea is not supported by a close examination of the facts. For example, incidence of breast cancer in native Japanese women and in first-generation immigrants to the United States is low. But the incidence of breast cancer in second-generation Japanese immigrants to the United States approaches that of other American women. Similarly, the incidence of breast cancer in black African women is low, but the incidence of breast cancer in black American women is similar to that of white American women.

Therefore, differences in the international incidence of breast cancer suggest that breast cancer may result from exposure to environmental carcinogens. However, the nature of these carcinogens is unknown. Generally, the incidence of breast cancer is highest in those countries with an affluent Western life-style. The highest incidence of breast cancer is found among women in The Netherlands, one of the most prosperous countries in the world. However, the low incidence of breast cancer in Japanese women seems to contradict a straightforward association between a highly industrialized society and a high incidence of breast cancer.

Contrary to the trend in most other cancers, breast cancer is not a cancer that predominates in women of poverty. In fact, breast cancer is more prevalent among professional and managerial level women than it is among women who

are manual laborers. This difference in incidence may be related to the fact that professional women often postpone marriage and childbearing until later in life than women who are manual laborers. Single women are more likely to get breast cancer than married women, and the age at first pregnancy is a very clear risk factor. Women who bear their first child at age 40 are four times more likely to contract breast cancer than women who bear their first child at age 20. These trends suggest that breast cancer incidence is likely to increase sharply in the future, because of the trend of delayed childbirth that became common as more women entered the work force in the 1970s and 1980s. In 1991, *USA Today* reported that 20% of 35-year-old women had never given birth.

Nulliparous women, or women who have borne no children, are generally at a greater risk of contracting breast cancer than are parous women. However, parous women who bear their first child after the age of 30 are at a greater risk than nulliparous women. Generally, the incidence of breast cancer declines in women who bear five or more children, but this relationship is clouded by the fact that most women who bear five or more children had their first child at a young age. There appears to be no association between breast cancer and the number of spontaneous or induced abortions. Recent data show that in populations in which frequent and long-term breast feeding is the norm, the risk of breast cancer is substantially reduced,¹³² raising the possibility that lactation is protective.

There are strong indications that female hormones play an important role in development of breast cancer. Women who suffer ovarian dysgenesis, a congenital failure of the ovaries to develop normally, generally do not contract breast cancer. Women who begin to menstruate at an early age are at a greater risk than women with a delayed menarche. Athletic activity, which tends to delay menarche, also leads to a decreased risk of breast cancer. The greater the age of a woman at menopause, the greater is the risk of breast cancer. These associations, together with the strong associations between parity and breast cancer risk, suggest that there is an underlying relationship between breast cancer risk and endogenous levels of the female hormones. Consistent with this idea is the fact that high doses of diethylstilbestrol (DES), a compound related to estrogen, have been implicated in development of human breast cancer. Experimental studies with animals have also shown an association between levels of estradiol in the blood and breast cancer risk. Yet, despite the simplicity of this idea, there are no conclusive data showing an association between hormone levels in the bloodstream and human breast cancer risk.

If there is an association between hormone levels in the bloodstream and breast cancer risk, then women who take oral contraceptives might be at a higher breast cancer risk than other women. Early studies of this question seemed to show that oral contraceptives did not lead to an increased risk of cancer, but these studies largely examined parous women who were taking contraceptives to control family size. In this group of women there was no association between oral contraceptive use and breast cancer, and oral contraceptives were, in fact, protective against benign breast disease. Yet parous women who had children at a young age are at a relatively low risk of developing breast cancer anyway. These early studies say nothing about the risk of breast cancer in young nulliparous women who take oral contraceptives to postpone their first pregnancy. Although several studies of the risk of oral contraceptives to nulliparous women are under way, there are as yet no clear-cut answers. The issue is very complicated because the dose level and composition of hormones in oral contraceptives have changed substantially over time.

Hormone replacement therapy is frequently used to relieve certain symptoms associated with menopause in some women. Estrogen replacement is also used to prevent osteoporosis and possible ischemic heart disease in postmenopausal women. If there is an association between estrogen levels in the bloodstream and breast cancer risk, then women who undergo hormone replacement therapy should be at a greater risk for breast cancer than other women. While existing clinical studies of this relationship do not provide a clear-cut answer, it seems likely that hormone replacement therapy does lead to a somewhat higher (i.e., less than 2-fold increased) risk of breast cancer. However, the risk of therapy is probably outweighed by the benefits, especially in those women prone to osteoporosis.

There are several additional risk factors that may be related to estrogen levels in the bloodstream. Obese women are at greater risk of contracting breast cancer than lean women, although this generalization seems to apply only to postmenopausal women. The mechanism of this increase in risk is unknown: it could be that diet accounts for the difference in risk, or it could be that the adipose tissue itself accounts for risk. A high-calorie diet in childhood can lead to earlier menarche, so that breast cancer risk may increase because of the early onset of puberty. It has also been hypothesized that certain intestinal bacteria are capable of converting bile acids into steroids, which then act as a precursor for estrogen. Another hypothesis proposes that conversion of a precursor molecule into estrogen occurs in adipose tissue, so more adipose tissue leads to

a greater conversion rate. There are also data suggesting that alcohol intake increases breast cancer risk, but the increase in risk is relatively small and the mechanism is completely unknown.

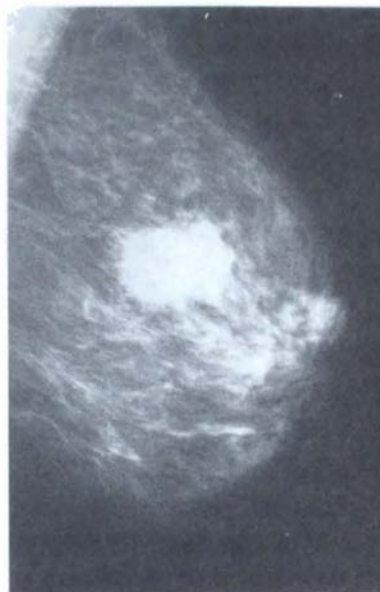
Radiation is probably the most significant environmental risk factor for breast cancer. Japanese survivors of the atom bomb attacks on Hiroshima and Nagasaki had an increased incidence of breast cancer 10 to 15 years after the radiation exposure. These women were particularly likely to develop cancer if they were exposed to radiation at less than 30 years of age. Although modern mammograms are unlikely to result in significant breast carcinogenesis, certain other medical procedures have been associated with an increased breast cancer risk. Women who receive radiation therapy to the thorax for an unrelated cancer have a higher risk of later developing breast cancer. Female tuberculosis patients who were treated by collapse of the affected lung often had this procedure done while X rays were used to visualize the lungs. These patients suffer an increased incidence of breast cancer 10 to 15 years after the initial exposure to X rays.

NATURAL HISTORY OF THE DISEASE

In 70 to 80% of breast cancer cases, the patient first presents to the physician with a hard lump or mass in the breast (Fig. 45). This lump is frequently (45% of cases) located in the upper outer quadrant of the breast, nearest the armpit. The next most frequent site for a lump is in the central portion of the breast, beneath the areola, or colored portion of the nipple (25% of cases). Usually the lump is painless, although many patients confess to slight discomfort at the site. Only a small percentage of women will actually experience pain at the site, so absence of pain should not be taken as a sign of a benign lesion. Even large, advanced breast tumors can be painless at diagnosis. About 75% of all breast lumps prove to be benign in nature. A mobile mass with well-defined margins in a woman under 30 is much more likely to be a benign fibroadenoma than a malignant tumor.

Other symptoms of breast cancer can include: pain in the absence of a palpable breast mass; discharge of fluid from the nipple; overt changes in nipple appearance; changes in the skin or contour of the breast; and enlargement of lymph nodes in the armpit. Nipple discharge is a fairly common symptom in women and seldom indicates serious disease, although it should always be referred to a physician. The most common type of discharge is the greenish-

Figure 45. An asymptomatic female patient (65 years o.d.) was routinely screened for breast cancer by mammography and a large lesion was noted in the right breast. There is a pattern of diffusely prominent milk ducts visible in this image, which was felt to be unusual for the age and parity of this patient. Images of the right and left breast are often examined together, so that the examining physician has an indication of how dense breast tissue is in the absence of tumor. Note the large dense mass in the upper part of the breast, which was proven by histological examination to be an infiltrating ductal adenocarcinoma. (Photograph courtesy of Dr. Julie Gandara and Dr. James A. Nelson, University of Washington School of Medicine.)



brown discharge associated with benign duct ectasia, a dilation of the mammary ducts caused by cellular debris. A clear discharge from one breast may indicate a benign tumor called an intraductal papilloma. A bloody or clear yellowish discharge, especially in women over 50, is more likely to be caused by an intraductal carcinoma.

Overt changes in the nipple are frequently associated with cancer. While benign dermatitis of the areola is fairly common in young women, this condition usually heals quickly upon treatment. Cancer of the breast may be associated with a dermatitis-like lesion that fails to respond well to treatment. Such a lesion may appear as a weeping, crusted, or scaly patch, resembling eczema, which does not heal in response to topical corticosteroids and has a very characteristic appearance when examined histologically. This type of lesion, called Paget's disease, is an uncommon but well-known sign of intraductal carcinoma. Any surface lesion of the breast, however small and innocent-appearing, should be biopsied if it does not heal promptly. Retraction of the nipple into the breast is characteristic of cancer if the retraction is progressive. However, congenitally flattened nipples and intermittent retractile nipples are both normal conditions, so nipple retraction is only worrisome if it is progressive or affects only one breast.

Changes in the overall contour of the breast should always be examined by a physician. Apparent enlargement or shrinkage of a breast, elevation or deviation to one side, or apparent flattening of a breast may all be signs of serious pathology. Changes in the texture of skin of the breast are also cause for concern, even without the obvious lesion of Paget's disease. Puckering or dimpling of the skin can indicate that an underlying tumor has invaded the skin, so that the skin is tethered to an underlying structure. More obvious changes include open ulceration of the skin or *peau d'orange*, a condition in which the skin resembles the rind of an orange. These conditions, when caused by cancer, usually indicate that the cancer is in an advanced stage. Any such skin lesion, even those that appear minor, should be biopsied immediately.

The presence of enlarged and painful lymph nodes in one or both armpits is a very common occurrence. Such enlargements frequently occur in the normal course of the menstrual cycle and can be associated with a range of other conditions including simple inflammation. However, abnormal or persistent lymph node enlargement can also be the first indication of cancer and should be evaluated by a physician.

Malignant transformation can affect either the epithelial cells lining the milk ducts or the secretory glands of the breast. When transformation affects only these cells, without breaching the basement membrane underlying the cells, this condition is referred to as carcinoma *in situ*. But carcinoma *in situ* appears to progress rapidly, and transformed epithelial cells can be invasive of surrounding tissue (Fig. 46). When the basement membrane is breached, so that malignant cells infiltrate the supportive tissue, or stroma, of the breast, this is referred to as invasive carcinoma. There are three different types of breast carcinoma *in situ* and approximately 20 types of invasive carcinoma of the breast, and the prognosis for each type is somewhat different. Generally, the prognosis is much more favorable for carcinoma *in situ* because it is less likely to have metastasized.

The incidence of pure carcinoma *in situ* is less than 4% of all new cases of breast cancer. But mammographic screening programs are successful at detecting breast cancers before they become palpable, so it becomes possible to detect carcinomas when they are still *in situ*. For example, in one large screening program, carcinoma *in situ* represented 26% of all detected tumors. About 59% of all pure *in situ* carcinomas are ductal carcinomas, while 28% are lobular carcinomas. Ductal carcinomas are carcinomas of the milk ducts of the breast, and it is not uncommon for such carcinomas to be multifocal, affecting more than one ductal system at the same time. The critical element for the

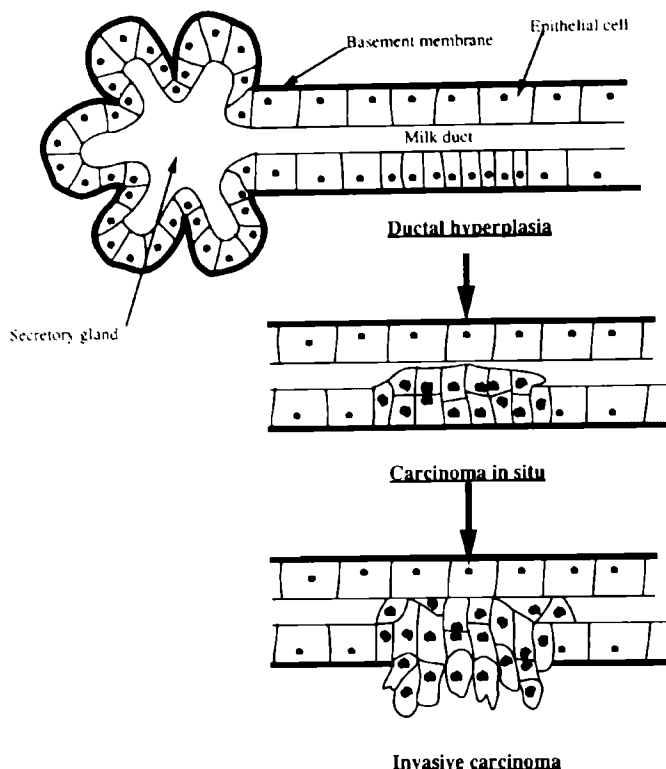


Figure 46. Schematic drawing of a milk gland and a milk duct, showing the basement membrane as a heavy line beneath the epithelial cells lining the structure (top drawing). Shown to the right in the milk duct is a small region of ductal hyperplasia. In the next diagram (middle) this hyperplastic region has become an early stage carcinoma *in situ*. A later-stage invasive carcinoma is shown (bottom) penetrating through basement membrane into surrounding breast tissue.

diagnosis of ductal carcinoma *in situ* is determining whether an intact basement membrane still surrounds the duct. Multicentricity and the existence of occult (undiagnosed) invasive carcinoma is probably the reason for the relatively high rate of recurrence of ductal carcinoma *in situ* after limited excision. Lobular carcinoma *in situ* is carcinoma that affects the cells of the secretory glands of the breast. The normal hollow structure of the secretory gland can be replaced by a solid mass of cells that, at least in carcinoma *in situ*, are still surrounded by the basement membrane.

Virtually all cancers of the breast (96%) are invasive at the time of diagnosis, and about 80% of invasive carcinomas are carcinomas of the milk ducts. Invasive ductal carcinoma is characterized by breaching of the basement

membrane around the duct, so that malignant cells no longer form a structure that resembles a duct. Tumor grade is determined by whether the tubular structure of the duct is preserved, the degree of pleomorphism of the cell nuclei, and the frequency of mitotic figures and hyperchromatic nuclei. The second most common type of invasive breast carcinoma is invasive lobular carcinoma, affecting the secretory portion of the milk gland. About 14% of new cases of invasive breast carcinoma are lobular carcinomas. Invasive lobular carcinoma may or may not be accompanied by foci of lobular carcinoma *in situ*. There are a number of different histological variants of this disease, so grading of this tumor is more difficult than grading of invasive ductal carcinoma. Approximately 5% of all invasive breast carcinomas are medullary carcinomas. These tumors are typically composed of densely packed, highly pleomorphic cells that are often heavily infiltrated with white blood cells. This type of breast tumor has a better long-term prognosis than the more common invasive ductal carcinoma, perhaps because the tumor often induces an immune response from the patient.

Generally the most important factor affecting patient prognosis in breast cancer is whether or not the tumor has metastasized at the time of diagnosis. The most common sites of metastatic spread are the lymph nodes in the armpit, liver, bones, skin, and lungs, although metastases may also be found in the adrenal glands, kidneys, ovaries, spleen, or thyroid.

Virtually all patients diagnosed with breast cancer undergo surgery to remove all or part of the involved breast. Until fairly recently, radical mastectomy was considered to be the sole therapeutic option for breast cancer. In this surgery, the entire breast is removed, together with the underlying pectoral muscles, and the lymph nodes in the adjacent armpit. In some cases, lymph nodes above the clavicle are also removed. This treatment is based on the idea that breast cancer begins as a single focus of disease that progresses in an orderly fashion, affecting an ever-enlarging field of adjacent tissue, and spreading through the lymphatic system to adjacent lymph nodes.

Breast cancer is now known to be, in many cases, multicentric at diagnosis. Disease may be localized for only a very brief time before it begins to disseminate through the circulatory and lymphatic systems. Consequently, removal of a large mass of tissue surrounding the tumor is unlikely to achieve a cure without further treatment, and the trend is now toward a type of surgery that is less disfiguring to the patient. This may mean that the primary tumor is removed, with only a small amount of surrounding tissue, in a procedure called a "lumpectomy." This procedure, often done in combination with radiation

therapy, is used to obtain local disease control. The tumor is usually assumed to have seeded distant metastases at diagnosis, even if these metastases are occult, and some sort of systemic therapy is used to obtain control of the metastases.

The question of how much breast tissue to remove during breast cancer surgery is one of the most controversial questions in modern oncology. Each surgical option, from the extended radical mastectomy, through the partial radical, to the lumpectomy, has strong proponents who quote studies that seem to demonstrate that one particular option is superior to all others. What is clear is that early, effective local treatment improves the odds of survival for the breast cancer patient. The goal of breast cancer surgery must be to obtain local control, while trying to assure freedom from metastases. Effective local control seems to decrease tumor metastasis and certainly leads to longer patient survival. In this light, cosmetic considerations of surgery should be secondary.

Radical mastectomy is unsurpassed for achieving local control of breast cancer. With meticulously performed radical mastectomy, local recurrence is near zero for patients with early stage disease, and only 3 to 6% overall. Therefore, the question becomes: to what extent can partial mastectomy or lumpectomy duplicate the degree of local control achieved by radical mastectomy? Studies with a 5-year patient follow-up after surgery often show little difference between any treatment option, but 5-year survival is not always a good predictor of 10-year survival in breast cancer patients. The International Union Against Cancer (UICC) analyzed nine different prospective, randomized clinical trials, all with a 10-year patient follow-up, involving a total of 5758 patients who received some degree of mastectomy. The UICC concluded that patients undergoing radical mastectomy enjoyed significantly better survival and better local control than patients undergoing lumpectomy, even if lumpectomy was combined with radiation therapy. This was true for early stage patients at both 10 and 20 years after treatment. Generally, the more complete the dissection of lymph nodes at mastectomy, the lower was the rate of local recurrence at 10 years follow-up, although this difference was not always significant in the separate studies. Other studies have shown that if local recurrence does follow radical mastectomy, it generally occurs within 3 years of surgery. In contrast, local recurrence after breast-conserving surgery occurs at a rate of about 2% per year for up to 14 years after surgery. This may be a compelling consideration for the woman who wishes to put the disease behind her and get on with her life.

While the current survival rates show limited success in treating breast cancer, it is an important point that current therapy can save many patients who

would otherwise die. A large, retrospective analysis was done of 1308 breast cancer patients, with tumors at all stages, who refused therapy. This analysis showed that the 5-year survival rate in the absence of treatment was 19%, while the 10-year survival rate was only 5%. In contrast, aggressive treatment of breast cancer patients, diagnosed with tumors at all stages, yields roughly a 66% 5-year survival rate, and a significantly better quality of life at all stages of the disease posttherapy.

BIOLOGY SPECIFIC TO THE TUMOR

Breast tumors are usually rather slow-growing tumors. It has been calculated that, starting when the first cell becomes malignant, it may take up to 7 or 8 years for a breast tumor to achieve a diameter of 1 cm. Therefore, breast cancer should be considered a chronic condition at the time of diagnosis. Breast tumors spread via the lymphatic system and the bloodstream, and the long lag time between initiation and diagnosis can give ample time for metastasis to occur.

A very important factor affecting prognosis in breast cancer patients is the presence or absence of hormone receptors in the tumor tissue. Tumor biopsies are now routinely screened for the presence and concentration of estrogen receptor (ER) or progesterone receptor (PR). The ER is capable of binding estrogen from the patient's bloodstream and transferring hormone into the cell, where it may modulate cell growth rate or gene expression. The ER binding capacity of a tumor is expressed in terms of the quantity of estrogen bound per unit of cell protein. Values above 10 (femtomoles per milligram cytosolic protein) are considered positive, while values of 3 to 10 are intermediate, and values less than 3 are negative. The degree of ER positivity is correlated with the grade of the tumor and can be predictive of whether the tumor is likely to respond to hormone manipulation. The ER value tends to increase with the age of the patient. In the majority of cases, the initial ER determination of a tumor remains unchanged throughout the course of the disease, and the ER values of the primary tumor and any metastases are likely to be similar. PR content of breast cancer cells is likely to be related to ER content or activity, but PR value shows no correlation with patient age.

Women with ER-positive tumors have a more favorable prognosis than women with ER-negative tumors, because ER-positive tumors tend to be dependent on estrogen availability for continued growth. A study of women

with advanced breast cancer showed that ER-positive tumors respond to hormone manipulation 60% of the time, while ER-negative tumors respond to such manipulation less than 10% of the time. If ER values are above 100 before therapy, this indicates a high probability of tumor regression following estrogen deprivation. Overall, the women most likely to respond favorably to breast cancer therapy are postmenopausal women with small ER-positive tumors that have not yet metastasized.

The precise role of female hormones in the carcinogenesis of breast cancer is unclear. Various roles for estrogen have been postulated, including: estrogen as an initiator, interacting with the cellular DNA and causing development of the malignant state; estrogen as a promoter, acting to promote the carcinogenic action of other carcinogens; or estrogen as permissive of various carcinogenic events. In any event, it is clear that hormones are involved in the growth of most mammary tumors and that hormone manipulation can induce breast cancer remission in some patients with breast cancer. Hormone manipulation is achieved in the clinic in several different ways (Fig. 36). Endocrine ablation can be achieved by surgical removal of the ovaries, the adrenal glands, or the pituitary gland. Endocrine supplementation may be obtained by treating the patient with estrogen, progestin, or androgen. An antiestrogen such as tamoxifen may also be used to inhibit the binding of estrogen to the ER. Alternatively, various agents (e.g., aminoglutethimide, Trilostane, Danazol, or various hormone analogues) can be used to inhibit estrogen synthesis.

A potentially important prognostic factor in breast cancer patients is whether or not the tumor has undergone gene amplification. Researchers at UCLA have studied a cellular oncogene (called *c-erbB-2* or HER-2/neu) that is amplified in some breast tumors. They showed that amplification of *c-erbB-2* could be predictive of clinical outcome, even in node-negative breast cancer patients. This oncogene tended to be amplified in patients who suffered a recurrence of the disease, while it was not amplified in patients with a favorable long-term treatment outcome. In fact, 18% of node-negative patients who relapsed had extra copies of *c-erbB-2*, while only 5% of patients who remained in remission had extra copies of this cellular oncogene. Amplification of *c-erbB-2* was a significant predictor of poor prognosis, and was especially good at predicting the length of time before relapse of breast cancer. Gene amplification was independent of most commonly used prognostic factors, and it had greater predictive power than other prognostic factors even though the test had low sensitivity.¹³³

Because early stages of breast cancer are far more treatable than advanced

stages, a key to increasing the cure rate for breast cancer is to identify patients at risk of developing the disease. Recent research has been directed toward identifying genetic factors that increase susceptibility to breast cancer, since genetic factors may be responsible for 5 to 10% of all cases of breast cancer. A recent study showed that a condition known as proliferative breast disease (PBD), which is a benign proliferation of epithelial cells in the milk ducts, may be a precursor to breast cancer in some women.¹³⁴ First-degree relatives of women with breast cancer were screened for PBD by fine-needle aspiration of breast tissue. About 35% of first-degree relatives of breast cancer patients had PBD, while only 13% of a normal population of women had PBD. This suggests that first-degree relatives of breast cancer patients are genetically predisposed to PBD, and that PBD may undergo progression from a benign condition to a malignant breast cancer. This does not mean that all women with PBD will develop breast cancer; in fact, most patients with PBD never develop any breast malignancy. But the results suggest that there may be a chain of events leading to breast malignancy and that PBD may be the first link in that chain.

Recently an association was found between breast cancer and a specific gene mutation. Researchers from the University of California identified 23 families with an inherited predisposition to breast cancer. Among these 23 families, there were 146 cases of breast cancer, with cases often diagnosed when the patient was young, or diagnosed in both breasts simultaneously, or diagnosed in male family members. A chromosomal analysis was performed on the 329 members of these families. It was found that one region of chromosome 17, which is normally a highly variable chromosome, was highly similar among the family members. Loss of genetic variability in this chromosomal region was associated with breast cancer. Apparently an as-yet-unidentified gene on chromosome 17 causes an inherited susceptibility to early onset breast cancer.¹³⁵

Interestingly, loss of a part of chromosome 17 is seen in 75% of patients with colorectal cancer, and is associated with deletion of the p53 tumor suppressor gene. There is now evidence to suggest that the involved region of chromosome 17 in breast tumor patients is also the p53 gene.¹³⁶ A very sensitive technique was used to test for mutations affecting p53 in 26 surgically excised primary breast tumors. It was found that 46% of these tumors showed mutations of the p53 gene, but only 30% of these tumors showed a loss of genetic information affecting chromosome 17. Therefore, loss of genetic information on chromosome 17 may be a later event in cancer development than mutations affecting p53, and mutations affecting p53 appear to be rather

common in breast tumors. It may eventually become possible to screen women for mutations of p53, to determine which women fall into a high-risk group for breast cancer.

An important feature of breast cancer is that in about half of the patients with breast cancer (13 to 74% in various studies), there is evidence that the cancer is multicentric at diagnosis. This means that there are microscopic foci of tumor in quadrants of the breast other than that in which the primary tumor was found. This accounts for why radical mastectomy is so frequently recommended as therapy, even when a tumor mass is small. But the clinical significance of multicentricity is fairly unclear. It is very unusual for multiple cancers in a single breast to become clinically overt, or for cancers to occur in both breasts simultaneously. This suggests that multicentric foci of breast cancer may not progress. In women over 70 years old who died of unrelated causes, the incidence of clinically occult intraductal carcinoma is 19 times higher than the reported incidence of this disease.¹³⁷ These occult tumors may be actively suppressed by the immune system of the body as long as the tumor burden is small, or they may actually be regressing for unknown reasons. There is some evidence suggesting that multiple steps are involved in the creation of an invasive or metastatic breast tumor, and occult tumors may simply be tumors that have not undergone the next step in the progression to malignancy.

CHAPTER 16

Liver Cancer

OCCURRENCE

Primary cancer of the liver accounts for only 1% of all malignant cancers in North and South America and in Europe, with about 13,000 new patients diagnosed in the United States in 1990. For much of the world, incidence of this cancer is only 2 to 5 per 100,000 members of the population. But in parts of Africa and Asia, liver cancer accounts for up to 30% of all cancers, making it regionally the most prevalent form of cancer. In Japan the incidence of liver cancer is 15 cases per 100,000 people, in West Africa 58 per 100,000, and in Mozambique 104 per 100,000. The West African incidence of liver cancer is roughly comparable to the incidence of lung cancer in the United States. The reason why occurrence of liver cancer is so strongly linked to geography is that viral hepatitis, epidemic in many parts of Asia and Africa, is a potent risk factor for this cancer.

In the United States, carcinoma of the liver is about four times more likely to affect men than women, with a peak incidence in the fifth or sixth decade of life.¹³⁸ In areas with a high prevalence of viral hepatitis, the peak incidence of liver cancer occurs 10 to 20 years earlier than in the United States. Cirrhosis, a progressive degenerative disease of the liver, is found in 60 to 80% of all primary liver cancer patients, irrespective of geography.

There are two principal types of liver cancer, but there is little utility in distinguishing between them, since the clinical course of both types is similar and tumors are often a mixture of the two. In any case, carcinomas that arise from cells of the liver itself are called hepatocellular carcinomas, while carcinomas that arise from the bile ducts are called cholangiocarcinomas. Both tumor types may be referred to as hepatoma, a nondescriptive term whose use should be avoided. Hepatocellular carcinoma accounts for about 80 to 90% of all liver cancers.

RISK FACTORS

One of the most important risk factors for liver cancer is exposure to the hepatitis B virus (HBV), which causes hepatitis, a chronic inflammation of the liver. In some parts of Africa, the prevalence of antibodies to HBV in the bloodstream is between 1 and 10%, indicating that this virus is nearly epidemic in certain populations. There is evidence that viral DNA actually integrates into the DNA of liver cells in patients with chronic HBV infection, and viral integration of HBV DNA is relatively common in cancer patients. In fact, most patients with hepatocellular carcinoma superimposed on chronic liver disease show clear evidence of HBV infection.

Several other chronic liver conditions predispose the patient to develop liver cancer. Cirrhosis of either the alcoholic or the postnecrotic type, is the most common precursor to hepatocellular carcinoma in the United States. Cirrhosis is a progressive disease of the liver, which results in loss of liver cell function, disruption of blood flow within the organ, and loss of normal organ architecture. Alcoholic cirrhosis probably results from direct toxicity of alcohol to liver cells, with cell death leading to regenerative growth of cells, the formation of fatty deposits, and development of large amounts of scar tissue within the liver. Postnecrotic cirrhosis is a form of cirrhosis that is characterized by necrosis affecting whole lobes of the liver, often as a result of viral infection, liver toxicity, or interrupted blood flow to the liver. Both types of cirrhosis result in cell death, so surviving cells are stimulated to proliferate to enable the liver to function normally. As we have seen, malignant transformation of cells is often associated with chronic stimulation of cell division.

Another chronic liver condition that can lead to liver cancer is a hereditary disease called hemochromatosis.¹³⁹ This is an iron-storage disorder in which an excess of iron is taken up from the digestive tract and deposited in the liver, pancreas, and heart. Hemochromatosis is associated with progressive and massive iron overload, with concentrations of iron in the liver and pancreas elevated 50- to 100-fold compared with normal, while the heart may have 5 to 25 times the normal quantity of iron. This causes progressive tissue injury, and organ failure can begin as patients reach 40 to 60 years of age. Hemochromatosis appears to be a relatively common genetic disease in white Anglo-Saxons, as about 10% of this population carries the gene but does not express the disease. Men are 5 to 10 times more likely to show clinical evidence of hemochromatosis than women, but severity of the disease appears to be moderated by several factors. About 30% of patients with hemochromatosis

develop hepatocellular carcinoma, but cancer appears to occur only in conjunction with cirrhosis.

In many parts of the world, exposure to dietary toxins is thought to play a significant role in development of liver cancer. The liver is the major organ of chemical detoxification, so it is likely to be exposed to any toxins inadvertently consumed in the diet. The most important dietary carcinogen is aflatoxin, a toxin secreted by a mold that commonly infects peanuts. Peanuts are an important part of the diet in parts of Africa and Asia, and aflatoxin may be present in large amounts in contaminated foods. Individuals can be continuously exposed to this carcinogen, which may act in conjunction with hepatitis virus exposure to dramatically increase risk of hepatocellular carcinoma. In addition, malnutrition may play a role in increasing the carcinogenicity of aflatoxin.

The sharply increased risk of liver cancer for men implies that there may be an excess risk conferred by exposure to male sex hormones. Alternatively, it may simply be that men are more likely to engage in high-risk behaviors such as chronic alcoholism. But the fact that men are also five- to ten-fold more likely to get hemochromatosis argues against such a simple interpretation. Hepatocellular carcinoma has been reported in some patients who received long-term androgen (male hormone) therapy. High levels of androgen receptors have been found in hepatocellular carcinoma tissue, whereas normal liver lacks these receptors. Although cancers of the breast, prostate, and ovary are associated with hormonal exposure, these organs are manifestly related to sex-specific functions, whereas it is difficult to imagine a sex-specific function for the liver.

Liver cancer has been induced in some patients by an unfavorable response to medical therapy. Thorium dioxide was an agent widely used in medical imaging until the mid-1950s. This agent is not excreted, but rather is stored in the liver for the lifetime of the patient. Thorium is a radioactive element, with an extraordinarily long biological half-life (400 years), so chronic low-level irradiation of the liver occurs in patients who received this drug. These patients often develop chronic liver disease or an unusual liver tumor called angiosarcoma after a 15- to 20-year latent period. Another medical treatment that has been implicated in development of liver cancer is long-term use of oral contraceptives. Such long-term use can lead to development of a benign liver tumor called a hepatic cell adenoma, but adenomas have occasionally been reported to undergo malignant transformation to hepatic carcinoma.

NATURAL HISTORY OF THE DISEASE

The liver has a very unusual pattern of blood flow, which can have important consequences for the liver cancer patient. Most organs are perfused by blood from a single major artery, which divides up into a single capillary bed. However, the liver has two major supplying vessels, which split up into two separate capillary beds. One of these major vessels, the hepatic artery, supplies blood to the liver in the same way that other organs are supplied with blood; the hepatic artery takes blood from the aorta and brings it to the liver while the blood is still rich in oxygen, glucose, and other nutrients. But the hepatic portal vein, the other major vessel supplying the liver, has a very different origin: it arises from capillary beds scattered throughout the organs of the abdomen. Blood from capillary beds in the spleen and the walls of the intestine is collected into the hepatic portal vein, then this blood flows to the liver and splits into a second capillary bed. This is unique, because there are actually two capillary beds one after the other, whereas most arteries supply only a single capillary bed. Having two capillary beds in series means that oxygen and glucose in the bloodstream could potentially be removed in the first capillary bed, so that blood reaching the second capillary bed is already depleted of nutrients required by liver cells. However, the purpose of the hepatic portal circulation is to bring nutrients absorbed in the intestine directly to the liver. Thus, blood in the portal circulation would be unlikely to be depleted of glucose and other nutrients, although oxygen could be depleted. The presence of the portal vein, which drains the small and large bowel, explains why hepatic metastases are so frequent in patients with colorectal carcinoma.

The diagnosis of liver cancer is typically a difficult one to make, because cancer usually occurs in patients with underlying liver disease, so that signs and symptoms of liver cancer are initially interpreted as symptoms of progression of cirrhosis. Enlargement of the liver (hepatomegaly) is a common symptom in more than half of liver cancer patients. Enlargement is often accompanied by pain or tenderness, usually of a moderate degree, and often localized to the upper or upper right portion of the abdomen. However, these symptoms can also be associated with cirrhosis, and are by no means diagnostic of liver cancer.

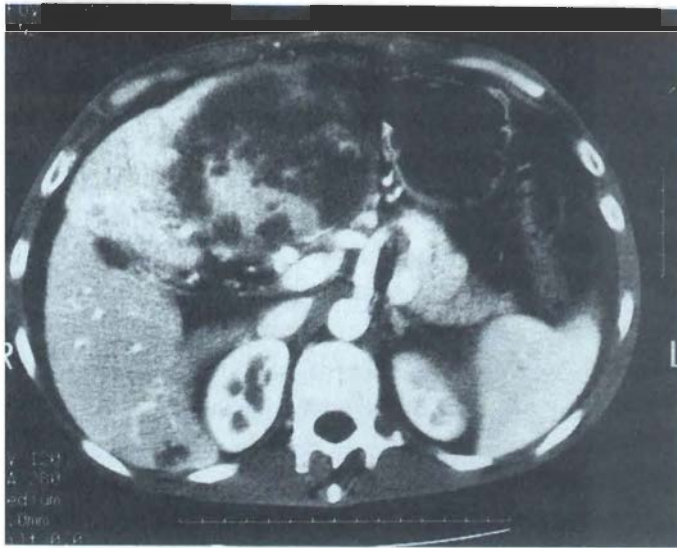
The liver cancer patient typically suffers from a feeling of general malaise, often coupled with symptoms of progressive obstructive jaundice. Jaundice is a serious condition in which the skin and whites of the eyes take on a yellowish cast because of deposition of a pigment called bilirubin from the

bloodstream. Under normal conditions, bilirubin does not accumulate in skin because it is excreted by the liver into the intestine (as bile). When a tumor causes impairment of bile secretion, or when movement of bile out of the liver is obstructed, large amounts of bilirubin accumulate in the liver and are excreted into the bloodstream.

Jaundice occurs when the main hepatic bile ducts are compressed or occluded, so this is primarily associated with cholangiocarcinoma, and jaundice is relatively uncommon in hepatocellular carcinoma in the absence of active cirrhosis. In fact, hepatocellular carcinoma can destroy up to 80% of normal liver tissue before symptoms of obstructed bile flow become obvious. Since the most frequent cause of obstructive jaundice is alcoholic cirrhosis, the physician must evaluate the jaundiced patient very carefully for symptoms specific to liver cancer. Making a differential diagnosis between cirrhosis and liver cancer is complicated by the fact that, in the United States, up to 15% of all patients with cirrhosis (or 3% of patients with alcoholic cirrhosis) eventually contract cancer of the liver, while in parts of Africa and Asia 40 to 50% of cirrhotic patients contract liver cancer.

To differentiate between cirrhosis and liver cancer, the physician must search for symptoms specific to liver cancer. Identification of a liver mass during palpation of the abdomen is strongly suggestive of cancer (Fig. 47), especially if the rest of the liver is not grossly nodular from end-stage alcoholic cirrhosis. Another clue to cancer is the presence of a rubbing sound within the abdomen when a stethoscope is used to monitor sounds made by the internal organs. Occasionally, the liver cancer patient presents to the physician with symptoms of general metabolic disturbance. These can include: polycythemia (an increase in the red blood cell count of the blood), hypoglycemia (a decrease in blood sugar), hypercalcemia (an increase in concentration of calcium ions in the blood), or acquired porphyria. Porphyria is an abnormality of liver metabolism that results in thickening and dark pigmentation of the skin, neurologic symptoms, and excretion of chemicals called porphyrins in the urine. These symptoms of metabolic disturbance result from loss of normal liver function, and can indicate that a tumor is relatively far advanced.

Liver tumors tend to be diagnosed rather late in their course, when tumor cells have already begun to spread into regional lymph nodes or to secondary sites in the liver. Only about 10–20% of all hepatocellular carcinomas are classified as early stage at diagnosis. Invasive growth into large branches of the portal vein occurs in 65% of all hepatocarcinomas, while invasion of the hepatic vein is seen in 23% of all hepatocarcinoma cases. Furthermore, seeding of



puted X-ray tomograph (CT) image of the abdomen of a 47-year-old male patient of severe upper abdominal pain and was examined for possible liver cancer. The very large primary liver tumor, visible as a dark area at the top center-left. The tumor diffusely infiltrating the liver; liver is visible as the large organ filling the entire left abdominal cavity at this level. Note that the kidneys appear as bright oval organs on either side of the spinal column at the bottom of the image, and ribs are visible as bright streaks at the periphery of the image. (Photograph courtesy of Dr. James A. Nelson, University of Wisconsin School of Medicine.)

Secondary liver tumors from a primary hepatocarcinoma is very common. Approximately 70% of all hepatocarcinomas are classified as advanced (Stage IV) at the time of diagnosis, and patients often present to the physician with a large tumor, ascites, and an abnormally low level of blood protein (hypoalbuminemia). Such patients have an average survival time of about 1 month.

The incidence of clinically significant metastatic disease to the liver is at least 10%, which is greater than the incidence of primary cancer of the liver. Hepatic metastases have been reported in 30–50% of all patients with end-stage colorectal cancers. Metastases to the liver are found in 20–25% of patients undergoing surgery for cancer of the colon or rectum, while liver metastases are found in 49% of pancreatic cancer cases, and 20% of stomach cancer cases.

Factors that influence liver cancer growth are important in that they determine the prognosis and resectability of the tumor.¹⁴⁰ Hepatocellular carcinomas can take the

form of a single nodule or multiple nodules, and they can spread along ducts or be diffusely invasive. Carcinomas that are single or multiple tend to be fairly well demarcated from surrounding liver tissue, while the spreading and invasive types are much harder to separate from normal liver. Tumor cells can assume a morphology resembling that of normal liver cells, or they can form masses of cells with varying degrees of anaplasia, including a form that bears no resemblance to normal liver cells. Cholangiocarcinomas tend to occur as solitary tumors along minor bile ducts, but about one-third of these tumors arise from cells near the major bile ducts. Portal bile duct tumors are usually small at the time of diagnosis because they occlude the bile duct early, resulting in bile obstruction and biliary cirrhosis. Cholangiocarcinoma cells also exhibit a wide range of anaplasia, but the grading of both hepatocellular carcinomas and cholangiocarcinomas appears to be of relatively little value in determining patient prognosis.

Surgery offers the only real hope of cure, yet surgery will not even be attempted in many patients because of the risk of death associated with surgery. Both cirrhosis and the factors that cause cirrhosis (alcoholism or chronic HBV infection) are contraindications for surgical resection of liver tumors. Other risk factors that mediate against liver surgery include: jaundice, diabetes, bacterial infection, malnutrition, weight loss exceeding 10% of body weight, and insufficiency of kidney excretion.¹⁴¹

Liver surgery is an exceedingly difficult art, because the blood supply to the liver is so complex and the organ itself is rather delicate. This is especially true of a cirrhotic liver, which may be prone to failure even in the absence of stresses such as surgery. The major types of liver surgery differ in that different parts of the liver are removed, and which particular surgery is used depends on the location and size of the tumor. For example, in a right lobectomy a very large portion of the liver is removed, but this can be tolerated in an otherwise-healthy liver, because the liver has a remarkable capacity to regenerate itself. In fact, up to 80% of the liver can be removed, and this will be compensated for by regrowth of liver within a few weeks in a healthy liver. But if liver function is impaired by an underlying disease, reduction in the volume of functioning liver by more than 50% is likely to result in acute or chronic liver failure. Surgical management of liver cancer is therefore a careful balancing act between obtaining an adequate clear margin around the tumor and leaving enough of the normal liver behind to prevent liver failure. The greater the amount of liver tissue removed, the greater is the risk of liver failure, so excision of large normal margins around the tumor may not be possible.

The liver has a segmental structure, with segments defined by the pattern of vasculature supplying the liver. It is often possible to remove a segment in which there is tumor without compromising function of the other segments. This segment-oriented approach has found favor recently because it lessens the risk of operative mortality or subsequent liver failure. It also is apparently better at obtaining a clear margin around the tumor. The segmental approach often employs intraoperative ultrasound to guide the surgeon during surgery, so that there is a constant feedback of information from the ultrasound instrument to the surgeon.

BIOLOGY SPECIFIC TO THE TUMOR

Screening programs for cancer of the liver have been in place since the early 1970s in some parts of the world that have a high incidence of disease. The intention was to screen large numbers of individuals, to identify patients before they begin to show symptoms, because resectability, operative mortality, and prognosis for asymptomatic tumors is far better than for symptomatic tumors. Because a large number of patients have been followed for a long period of time by hospitals doing liver cancer screening, new insight has been gained into the natural history of the disease. The best marker for primary liver cancer is an increase in blood levels of alpha fetoprotein (AFP), a protein that is present in 70–90% of all hepatocellular carcinoma patients. However, this protein is also found in patients with hepatitis, cirrhosis, biliary tract obstruction, and alcoholic liver disease, so it is of limited utility in screening for liver cancer.¹⁴² The time interval between the first detectable rise in blood levels of AFP and diagnosis of a still-asymptomatic tumor (4 cm or less in diameter) is about 10 months.¹⁴¹ After another 4 months, the tumor has likely increased in volume 5-fold (to a diameter of 9 cm), at which point the patient begins to show symptoms of liver cancer. In the absence of treatment, growth of the tumor is relentless and rapid, and death occurs after another 4 months. Thus, by the time most patients seek the care of a physician, the tumor is far advanced and the patient can undergo rapid deterioration. The success of screening programs in the Far East may indicate that such programs should be emulated in the United States, at least among certain high-risk populations.

Because of the unique pattern of blood flow to the liver, oxygen is far more likely to be in short supply than glucose. The liver receives about 25% of all of the blood pumped by the heart, with blood entering the liver from either the

hepatic portal vein or the hepatic artery. The hepatic portal vein supplies about 70% of the total blood flow to the liver but, because the portal circulation is depleted of oxygen as it passes through the intestine, it supplies only about 50% of the oxygen needed by the liver. The hepatic artery contributes only about 30% of the total blood flow to the liver, but it must supply the other 50% of oxygen required by the liver.¹⁴³ The unusual blood supply to the liver can have important consequences to the patient undergoing surgery, because all anesthetic agents reduce liver blood flow. During liver surgery, especially during resection of a liver tumor, massive blood loss is common, and this can cause hypotension, or an abnormally low blood pressure. This could potentially result in inadequate perfusion of all or part of the liver, leading to liver cell death. Furthermore, anesthetic agents are usually inactivated in the liver. The facts that blood flow to the liver may be reduced, that liver function may be impaired, and that portions of the liver may be removed during surgery, can make anesthetic effects more difficult to predict in liver cancer patients.

Tests of hepatic function are routinely done on the suspected liver cancer patient, to aid in diagnosis and to determine the ability of the patient to tolerate therapy. Hepatic function tests use blood samples that are analyzed for certain chemicals, to determine how well the liver is functioning. For example, the concentration of proteins involved in blood clotting might be measured, because protein synthesis can be impaired in liver disease, and this would impact upon the chances for successful surgery.¹⁴⁰ Measuring blood levels of gamma globulin can inform the physician about the ability of the body to respond to infection. There are more than a dozen hepatic function tests that can be done, but these tests are not specific to cancer because hepatic function is also impaired in patients with cirrhosis.

HBV is a potent liver carcinogen, because overproduction of a viral protein initiates a process of liver cell injury, inflammation, and regenerative hyperplasia. This places large numbers of liver cells at risk for mutation, since so many cells are undergoing mitosis. Regenerative hyperplasia tends inexorably to progress to hepatocellular carcinoma. Regenerative hyperplasia may be a general mechanism of liver carcinogenesis, operative in cirrhosis as well as HBV infection.¹⁴⁴

Recently there has been an explosion of activity among researchers studying tumor suppressor genes. Tumor suppression is associated with several genes, but the p53 gene has already been documented as the most frequently mutated gene in human cancer. There are at least 30 different mutations known to affect p53 gene expression, all of which can be carcinogenic. Mutation of the

p53 gene occurs at high frequency in hepatocellular carcinoma patients from China and southern Africa, where aflatoxin is a major risk factor for cancer.¹⁴⁵ Mutations of the p53 gene were detected in 65% (17 of 26) of cases of multiple hepatocellular carcinoma, suggesting that p53 mutation is very common in aggressive tumors.¹⁴⁶ However, evidence is accumulating that mutation of the p53 gene alone is not sufficient to cause liver cancer, since two different tumor suppressor genes are often eliminated in the course of tumor formation.

The best-known tumor suppressor genes, p53 and the retinoblastoma (RB) gene, both are growth-suppressing proteins, and they appear to serve complementary functions in growth regulation. A potential interaction between mutations of p53 and RB has been identified in hepatocellular carcinoma. The p53 gene was examined in 43 primary hepatocellular carcinomas: 22 advanced cancers and 21 early stage cancers. Abnormalities of the p53 gene were detected in at least 36% (8 of 22) of advanced tumors, but in none of the early cancers. Loss of the RB gene was seen in 6 of the 8 tumors with a p53 mutation, suggesting that loss of both tumor suppressor genes may be involved in progression of hepatocellular carcinoma.¹⁴⁷ Mutation of both the p53 and RB genes has also been observed in small-cell lung carcinoma and soft tissue sarcoma.

Some recent research has focused on the possibility that liver cancer might be treated with antibodies directed against a protein common to many liver tumor cells. Experiments were performed on a human hepatoblastoma cell line implanted in a “nude” mouse, a strain of mouse that has an incompetent immune system. Absence of a functional immune system in these mice permits human tumors to grow in mouse tissue, whereas mice with a normal immune system quickly reject transplanted human tumor cells. Nude mice with human tumors were treated with antibodies directed against a protein called ferritin, which is involved in transport of iron into cells. These antibodies were attached to radioactive atoms, so that when antibody bound to a cell, the bound cell was killed by radioactivity. Under certain conditions, animals treated with radioactive antibodies had a significantly longer survival than untreated controls, and 50–75% of the animals appeared to be cured of their tumors. Thus, radioactive antibodies can deliver large, possibly curative doses of radiation to a human tumor implanted in a mouse.¹⁴⁸

A series of very controversial papers has reported on the success of treating advanced hepatocellular carcinoma patients with radioactive antibodies. Researchers have reported that most human hepatocellular carcinoma cells express ferritin on the cell surface. A series of 105 hepatocellular

carcinoma patients were treated with radioactive antibodies to ferritin, combined with radiation therapy and chemotherapy. An evaluation of 66 patients from this group was reported; 24 of these patients showed a partial response to therapy, while 4 patients showed a complete response.¹⁴⁹ This work has been widely criticized, because no data were reported for 39 of the original patients entered in the protocol, raising the possibility that more than a third of the original patients were deleted from the study for unspecified reasons. More recently, striking successes were again reported by the same group; 11 patients with unresectable hepatocellular carcinoma were treated with radioactive antibodies to ferritin. Of these 11 unresectable cancers, 7 were converted to resectable cancer, and 6 of these patients were reported to have a complete tumor resection.¹⁵⁰ It remains to be seen whether these encouraging results can be replicated by other researchers, and whether this use of biological therapy will become clinically accepted.

CHAPTER 17

Cancers of the Stomach and Esophagus

OCCURRENCE

Gastric cancer is probably the most common lethal malignancy in the world, with over 680,000 new cases diagnosed worldwide each year. It is very common in Japan (where it accounts for more than half of all cancer deaths), in the peoples of the Andes in both Central and South America, and in parts of eastern Europe. In Japan, the mortality rate from gastric cancer is as high as 60 per 100,000 members of the population, and the disease tends to strike younger patients than elsewhere in the world, whereas the mortality rate in the United States is only 6 per 100,000. Despite the prevalence of gastric cancer in the rest of the world, its incidence has fallen progressively in the United States for about 50 years. But there were more than 23,000 patients diagnosed with gastric cancer in the United States in 1990, a number that constitutes about 2% of all newly diagnosed cancers in the country that year. More than 90% of gastric cancers are adenocarcinomas, derived from the epithelial cells lining the stomach. Of those gastric cancers that are not adenocarcinomas, about 5% are malignant lymphomas, or tumors of lymph tissue, while the remainder are leiomyosarcomas, carcinoid tumors, or squamous cell cancers that actually originated in the esophagus.¹⁵¹

Gastric cancer is typically a disease of later life, with the mean age at diagnosis in the United States being about 60 years old, and fewer than 5% of patients are diagnosed under 40 years of age. Gastric cancer tends to afflict men more frequently than women, with a male-to-female incidence ratio of 2:1, and blacks are generally more likely to develop gastric cancer than whites. Gastric cancer was once the most common cancer among men, until the dramatic increase in lung cancer that began 50 years ago. A decline in incidence of gastric cancer over the same time period has made it now the fifth most common cancer among men. Over the years during which the incidence of

gastric cancer has declined, the death rate for this cancer also declined by about 76%. Even in the short period of time between 1973 and 1987, the incidence of gastric cancer declined by 21%, while mortality declined by 29%.¹⁵²

Esophageal cancer is less common than gastric cancer, but it generally has a poorer prognosis. This cancer is also more common in other parts of the world than in the United States, with a particularly high disease incidence in parts of China, Iran, and Russia. In certain provinces of China, esophageal cancer afflicts 130 per 100,000 population, while in Japan the disease incidence is 46 per 100,000, and in the United States the disease incidence is only 10 per 100,000. Nonetheless, in 1990 there were nearly 11,000 cases diagnosed in the United States, which accounted for about 1% of all of the newly diagnosed cancers that year. Esophageal cancer, like gastric cancer, is generally more likely to affect men than women, although in Sweden and Finland men and women are equally afflicted. About 90% of all esophageal cancers are squamous cell carcinomas, with adenocarcinomas making up most of the remaining 10% of cases. Rare cancers of the esophagus include carcinosarcoma, melanoma, pseudosarcoma, and occasionally an adenocarcinoma of the stomach that extends into the esophagus.

RISK FACTORS

Over the past 50 years the annual mortality from gastric cancer in the United States has fallen from 25 to 6 per 100,000 population. A similar but lesser decline is also evident in western Europe and, more recently, even in Japan. A great deal of attention has been focused on this decline in gastric cancer incidence, because it is both large and unanticipated. However, the reason for this striking decline is unknown.

The major cause of gastric and esophageal cancer is suspected to be diet. But comparative studies of diet in different cultures have failed to reveal any one food associated with a modified risk of gastric cancer. Instead, a general pattern of food consumption was found to be associated with an elevation or reduction of cancer risk. High-risk nutrition is characterized by a low proportion of animal fat and protein, together with a high proportion of hard-to-digest carbohydrates such as grains. A diet rich in foods that are highly salted to prevent spoilage, or containing a high proportion of smoked meat and fish, or rich in fava beans can increase cancer risk, as can a diet with a low proportion of fruits and vegetables.¹⁵³ It is noteworthy that a diet low in animal fat and

protein is a risk factor for gastric cancer, while it appears to be protective for colorectal cancer. One study of Japanese immigrants to the United States showed that as the incidence of gastric cancer declined over two generations after immigration, the risk of colorectal cancer increased.

Dietary factors that protect against gastric cancer include a diet rich in vitamins C and A, with a high proportion of fresh fruit and vegetables, and a high consumption of proteins and fat. Additional protective factors include careful food preparation, deep-freeze preservation of food, and a drinking water supply low in nitrates. The decline in gastric cancer incidence in the United States occurred coincident with a decline in the consumption of smoked, pickled, and salted foods. Gastric cancer incidence may thus have decreased because of the introduction of refrigeration as a means of food preservation. Similarly, the recent decline in gastric cancer in Japan is correlated with a reduction in consumption of pickled meat and more widespread use of refrigeration.

Oral administration of a chemical called nitrosoguanidine has been shown to induce gastric cancer in certain experimental animals. It has been suggested that gastric cancer may be associated with consumption of dietary nitrates, which are converted to nitrites in the stomach, and which can react with amino acids to form nitroso compounds similar to nitrosoguanidine. Nitrates may be present in drinking water, and nitrites were once widely used as food preservatives, so dietary exposure to these chemicals can be common. Yet a clear-cut link between nitrates, nitrites, nitrosamines, and gastric cancer has never been shown in humans, even though nitrites were banned as a food preservative in the United States because of the suspected link to gastric cancer.

Elevated consumption of alcohol has been implicated as a risk factor for both gastric and esophageal cancer, and there is an association between alcoholism and increased incidence of esophageal cancer. In the United States heavy consumption of beer is associated with a 10-fold increase in the incidence of esophageal cancer, while heavy consumption of whiskey is associated with a 25-fold increase in cancer risk.¹⁵⁴ It has been calculated that the incidence of esophageal cancer in the United States would be reduced about 80% if smoking and alcohol consumption ceased. However, studies of alcohol consumption in other countries have failed to confirm the connection between alcohol consumption and esophageal cancer, or have suggested that the connection is more likely to be the result of contaminants in alcohol. Recent studies have shown that consumption of very hot tea, or of a beverage called maté, drunk at a very high temperature by peoples of South America, is associated with an increased

risk of esophageal cancer. In general, there appears to be an association between esophageal cancer and the intake of substances that damage cells of the esophagus, suggesting that induction of an increased rate of cell division is the basic mechanism of esophageal carcinogenesis. Patients with esophageal burns from swallowing lye are prone to develop esophageal cancer, which is consistent with the general hypothesis that cell damage is associated with carcinogenesis.

Cigarette smoking has been implicated as a risk factor for both gastric and esophageal cancer. Smoking also increases the risk of cancers of the mouth, pharynx, larynx, lung, kidney, and bladder, so it is not surprising that it also has an effect on the esophagus and stomach. The relationship between smoking and esophageal cancer was explored by analyzing tissue collected from the esophagus of smokers and nonsmokers. Samples of esophageal tissue were collected during autopsies of 1202 American men with known smoking histories and these tissues were examined histologically for cancerous and precancerous conditions.¹⁵⁴ In the nonsmokers, only 6.6% of esophageal tissue samples showed atypical cell nuclei, and there were no cases of carcinoma *in situ*. In the smokers, atypical nuclei were found in 79.8% of tissue samples, and 15 cases of carcinoma *in situ* were identified (1.9% cancer incidence). The epithelial layer of cells in the esophagus tended to be thickened in smokers, reflecting a cell hyperplasia similar to that observed in the lungs of smokers. Although the link between smoking and gastric cancer is weaker than the link between smoking and esophageal cancer, an increased incidence of gastric cancer has been seen in coal miners who smoke and are exposed to coal dust.

Familial or hereditary conditions may play a role in the incidence of gastric cancer, and supposedly there were multiple gastric cancers in Napoleon's family. Gastric cancer is two to four times more common in persons with first-degree relatives afflicted with the disease, and identical twins are more likely to share the disease than fraternal twins. This suggests a genetic component of the disease, but no definitive proof of a hereditary predisposition to gastric cancer has been obtained. However, certain hereditary precancerous conditions are known to be associated with an increased risk of gastric cancer. Gastritis, or inflammation of the stomach, is associated with atrophy of the mucus membranes of the stomach and metaplastic or dysplastic changes in the cells of the stomach wall. Studies in China suggest that gastritis of long duration can lead to stomach cancer, particularly when combined with dietary exposure to nitrates and nitrites.

Certain other medical conditions are also associated with an increased risk

of gastric cancer. Achlorhydria is a condition in which there is no hydrochloric acid in the digestive juices secreted by the stomach. Patients with achlorhydria are 4 to 5 times more likely to have gastric cancer than people with normal stomach acid production. Patients with pernicious anemia, a progressive wasting disease of older adults associated with chronic shortage of vitamin B₁₂, are 18 times more likely to have gastric cancer. Finally, patients with acanthosis nigricans, a skin disease characterized by a proliferation of warty, purple growths on the armpit, neck, and groin, are more prone to several different cancers, including gastric cancer. However, gastric polyps and peptic ulcers are not considered to be risk factors for gastric cancer.

Studies of the incidence of esophageal cancer in China show that 25 to 60% of patients with esophageal cancer have first-degree relatives who also have this cancer. However, both members of such a family would typically live together in the same environment for more than 20 years, so a common exposure to environmental carcinogens may be more likely than genetic factors as a cause of cancer. An early study of two families in England, which between them had 18 cases of esophageal cancer in only three generations, showed that esophageal cancer can be associated with a medical condition called palmoplantar keratosis.¹⁵⁴ Palmoplantar keratosis is a hereditary condition in which there is hypertrophy of the horny layer of skin on the palms of the hands and the soles of the feet. This leads to the formation of variable, scaly, rough, red plaques of thickened skin. Of the 18 patients with esophageal cancer, 17 had preexisting palmoplantar keratosis, and the one remaining esophageal cancer patient may have had keratosis as well. Of the remaining 86 family members in whom keratosis was not found, there was only one case of esophageal cancer. Thus, keratosis may be inherited as a dominant genetic trait, and these patients may be predisposed to develop esophageal cell dysplasia. However, recent studies have failed to confirm a clear connection between esophageal cancer and other hereditary conditions.

Incidence of esophageal cancer is elevated in patients suffering certain inflammatory disorders of the esophagus, with the clearest connection established between esophageal cancer and a type of esophagitis called Barrett's esophagitis. The latter is a chronic ulcerative condition of the lower part of the esophagus, which may result from chronic reflux, or regurgitation of the contents of the stomach into the esophagus. In Barrett's, inflammation is associated with cell metaplasia (Fig. 1), as the flattened cells of the normal esophagus are replaced by columnar cells. These columnar cells are more typical of the cells lining the gastric cardia, where the esophagus opens into the

stomach. Barrett's esophagitis leads to esophageal adenocarcinoma in 2 to 5% of cases, so this is a very significant risk factor for a type of esophageal cancer that is normally somewhat unusual.

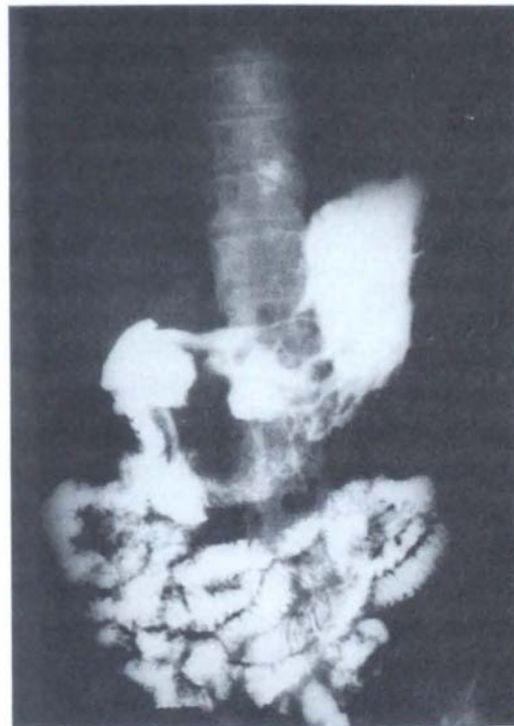
NATURAL HISTORY OF THE DISEASE

The most common complaint of the gastric cancer patient at diagnosis is upper abdominal discomfort, which can vary from mild discomfort after eating to severe, steady pain.¹⁵⁵ Abdominal tenderness is present in about one-third of patients, and one-quarter of patients present with symptoms that are classically ulcer symptoms, including burning abdominal pain, nausea, vomiting, and weight loss. Weight loss is observed in at least one-half of patients with gastric cancer, and severe weight loss, or anorexia, may be present before the patient seeks medical attention. Dysphagia, or difficulty in swallowing, may be experienced by some patients, and nausea and vomiting are relatively common, particularly in advanced disease. General weakness, vomiting of blood (hematemesis), passage of dark-colored, tarry stools (melena), and other alterations of bowel habits are common presenting symptoms in the gastric cancer patient. Iron-deficiency anemia is present in about two-thirds of patients at diagnosis, because of occult bleeding of the tumor, and occult blood in the stool is demonstrable in up to 80% of patients (Fig. 48).

Certain symptoms indicate that a tumor is far advanced and perhaps metastatic. Symptoms of advanced disease can include: distension of the abdomen resulting from spread of the tumor into the abdominal cavity (malignant ascites); enlargement of the liver; jaundice caused by obstruction of the bile ducts with metastatic tumor; pain from bone involvement; neurologic symptoms associated with metastases to the brain; shortness of breath from metastatic involvement of the lungs; or pelvic pain from ovarian spread. Obstruction of the bowel can indicate a large primary tumor or metastatic spread of the tumor to membranes lining the abdominal cavity. An abdominal mass is palpable in a minority of patients, and is usually associated with a poor prognosis.

The length of time that patients endure symptoms before seeking the help of a physician is variable, but averages about 6 months, and symptoms of several years' duration are not unusual. In North America, fewer than 10% of patients present to the physician with symptoms of early gastric cancer. In Japan, where screening efforts for gastric cancer are well established, it has

Figure 48. Abdominal X ray of a 60-year-old male patient who was examined to determine the cause of hematemesis, or vomiting of blood. Taken 3 hours after consumption of a barium contrast agent, the film clearly demonstrates a large lesion in the lower portion of the stomach. The image shows a very large ulcerated region, apparent here as streaks (irregularities in the gastric wall) in the lower middle portion of the stomach. This is consistent with a diagnosis of gastric carcinoma, although other diagnostic possibilities cannot be excluded on the basis of this film alone. The spinal column is visible behind the stomach, and the small intestine is coiled at the bottom of the image. (Photograph courtesy of Dr. Charles Rohrmann and Dr. James A. Nelson, University of Washington School of Medicine.)



been found that most patients with early curable gastric cancer are asymptomatic.

Gastric cancers are usually adenocarcinomas, but more specific tumor classification by microscopy is often impossible, because tumors can show a mixed morphology. Microscopic classification has little or no prognostic value anyway, and the only significant prognostic factors are the depth of tumor invasion into the gastric wall, the extent of lymph node involvement, and the presence of distant metastases. Gastric tumors usually spread by direct invasion through the stomach wall, and invasion can involve the esophagus, pancreas, colon, or liver. Lymph node involvement is rather common, and gastric tumors can also spread via the lymph circulation. Metastases of tumor into the abdominal cavity are observed in about 20% of patients, and blood-borne metastases to the liver are observed in about 30% of cases. Metastatic involvement of the lungs, brain, or other organs is much less common.

The symptoms of esophageal cancer are rather similar to the symptoms of gastric cancer, although dysphagia is more common in esophageal cancer patients.¹⁵⁶ Dysphagia, or difficulty in swallowing, begins with solid foods, but

an eventually progress to include liquids. In addition, rapid and profound weight loss is common in esophageal cancer patients, because of the dysphagia. Chest pain occurs as the tumor spreads to tissues surrounding the esophagus, and tumor invasion can cause esophageal bleeding. Bleeding from an esophageal tumor is usually slow, but the tumor can invade the aorta and cause rapid exsanguination of the patient. Esophageal obstruction can result from tumor growth, particularly when the tumor causes a napkin ring-like stricture of the esophageal walls (Fig. 49). Pneumonia, aspiration of fluid into the lung, or lung abscess can result from esophageal obstruction, but the most striking finding on initial examination is usually abrupt and recent weight loss. Symptoms of advanced disease include lymph node involvement and enlargement of the liver from metastatic spread of disease. Abnormal elevation of calcium in the bloodstream can result from ectopic hormone production by the tumor, since a hormone resembling parathyroid hormone can be released by esophageal tumors.



Figure 49. X ray of an 80-year-old male patient who complained of difficulty in swallowing. Several minutes after drinking a barium contrast agent, the esophagus is clearly seen in this image, leading down to the stomach. An annular constriction is apparent at the midpoint of the esophagus, with an irregular region below the constriction suggesting ulceration of the esophageal wall. The dark mass projecting into the esophagus below the constriction shows a large tumor mass, later identified as an ulcerating carcinoma. (Photograph courtesy of Dr. Charles Rohrmann and Dr. James A. Nelson, University of Washington School of Medicine.)

Development of esophageal cancer probably takes more than 20 years, with a long precancerous phase characterized by mild hyperplasia of cells lining the esophagus.¹⁵⁷ Yet cancer of the esophagus is usually advanced at diagnosis, and esophageal cancers are often invasive of surrounding tissues. In a series of 253 esophageal cancer patients diagnosed with Stage I disease, only 39% of patients had disease that would be described as carcinoma *in situ*, while nearly 61% of the cancers were locally invasive. Esophageal tumors can also spread indirectly through the lymphatic system, and this route of metastasis appears to be used relatively early in the course of tumor growth. A large study of esophageal cancer has shown that 42% of tumors are metastatic to regional lymph nodes at the time of surgery, with the most frequently involved nodes in the thorax. Various studies have shown that metastasis via the bloodstream is common in patients with advanced disease. Overall, lung involvement is seen in 32% of cases, and liver involvement is seen in 30% of cases.¹⁵⁷

BIOLOGY SPECIFIC TO THE TUMOR

Contrary to expectation, those countries with a higher incidence of gastric cancer, such as Japan, tend to have a considerably lower rate of mortality from the disease. For example, in Germany and the United States, where the cancer is relatively uncommon, the mortality rate is more than 80%, whereas in Japan it is about 50%. The reduced mortality rate in Japan could be the result of the extensive screening programs in place for gastric cancer. However, there is evidence to suggest that there are really two types of gastric cancer, although both types can be histologically classified as adenocarcinomas.¹⁵³

The expansive or intestinal type of gastric cancer is more common in men than women, typically strikes older patients, and is associated with a relatively good prognosis. A familial predisposition for this cancer cannot be confirmed, but exposure to environmental carcinogens may be important. This type of gastric tumor tends to be organized into glandlike structures and tumor cells typically are not invasive into the gastric wall. Expansive gastric cancer predominates in Japan and other countries with an elevated incidence of gastric cancer, and the recent worldwide decline in gastric cancer is probably related to a decrease in this cancer.

The infiltrative or diffuse type of gastric cancer is equally common in men and women, typically affects younger patients, and is associated with a poor prognosis. Evidence is fairly good that there is a familial predisposition to this

type of gastric cancer, and cancer incidence is linked to the presence of blood group A. Infiltrative gastric tumors contain cells that do not form glandlike structures, but rather are invasive into the stomach wall. Incidence of the infiltrative type of gastric cancer is roughly the same throughout the world, rather than showing the uneven geographic distribution of the expansive type of gastric cancer. It is this type of cancer, without an overlying incidence of expansive gastric cancer, that apparently predominates in the United States and in certain other countries, where the overall incidence of gastric cancer is low.

From a clinical standpoint, the most important difference between the infiltrative and expansive tumor types is that the expansive tumor seldom spreads more than a few millimeters into adjacent normal gastric wall. On the other hand, the infiltrative type of gastric cancer can spread up to 8 cm into the surrounding gastric wall, meaning that much larger tumor margins must be taken during surgery in order to remove all of the tumor cells. Extension of the invasive tumor type into surrounding tissues often takes the form of discontinuous islands of tumor cells.

Recent study of the genetic material from gastric cancer cells removed by surgery has shown that the quantity of cellular DNA can predict patients prognosis; generally, there is a poor prognosis for gastric carcinoma patients with aneuploid tumors. Tumor samples from 493 gastric carcinoma patients were analyzed and correlated with clinical findings. Of the 493 patients, 37% had tumor cells with a normal (diploid) quantity of DNA, while the remaining 63% of tumors were aneuploid. Tumor cell ploidy was one of the most important predictors of patient prognosis, along with the presence of metastases in the liver or the abdominal cavity, and the extent of lymph node involvement. Relative risk of death was 2- to 3-fold higher in patients with aneuploid tumors, and the most favorable prognosis was seen in patients with tumors composed of diploid cells with a small fraction of dividing cells.¹⁵⁸

The proliferation of normal cells is regulated by growth-promoting cellular oncogenes, whose effects are counterbalanced by growth-constraining genes called tumor suppressor genes. Genetic mutations that inactivate a tumor suppressor gene may make that gene unable to oppose the growth-promoting effect of a normal cellular oncogene, or of an oncogene introduced by a virus. The p53 tumor suppressor gene has been documented as the most frequently mutated gene in human cancer; at least 30 different mutations are known to affect p53, with the various mutations producing proteins that range from mildly dysfunctional to strongly carcinogenic. Scientists have investigated the frequency of mutation or structural alteration of p53 in samples of gastric

cancer and in gastric and esophageal tumor cell lines. No mutations were detected in 19 primary gastric cancer tumors, yet 2 of 4 gastric cancer metastases, and 4 of 8 cell lines established from gastric cancers, showed mutations of the p53 gene. This suggests that p53 mutation occurs only in the advanced stages of gastric cancer and that mutation may be important in gastric cancer progression or the survival of gastric cancer cells in cell culture. None of thirteen esophageal cancers showed p53 mutations (including 2 cell lines established from metastases).¹⁵⁹ However, another study showed that 48% of esophageal cancers had mutations affecting the retinoblastoma (RB) gene.¹⁶⁰ The absence of p53 mutations (and the presence of RB mutations) in esophageal cancer indicates that this is a very different disease than gastric cancer.

There is now broad acceptance of the idea that carcinogenesis is a multistage process. Cancer is thought to result from the combination of a tumor initiator, which causes an irreversible mutation in the affected cell, followed at some point by a tumor promoter. Exposure to an initiator could potentially occur at very low levels of exposure, so a tumor might not be produced for many years, although initiated cells are nevertheless primed for later exposure to a promoter. A tumor promoter is thus any agent that enhances proliferation of the initiated or mutated population of cells. Exposure to the promoter can result in formation of precancerous lesions, each of which can then progress to form a malignant tumor. Numerous experiments have shown that, if exposure to the promoter occurs before exposure to the initiator, no tumors develop, so carcinogenesis occurs only if initiation precedes promotion.

A model of multistage carcinogenesis has been developed for esophageal cancer, based on careful evaluation of patients in a part of China where disease incidence is extremely high.¹⁵⁴ Although this model is rather specific to the Chinese population of Linxian county, the model is a useful demonstration of multistage carcinogenesis and of the risk factors relevant to esophageal cancer. People of Linxian (China) often suffer chronic dietary deficiencies of vitamins A, B₂, and C, and this may predispose patients to development of esophageal cancer. When esophageal cells are exposed to either fungal infection or carcinogens in the food supply, the result can be esophagitis, a chronic inflammatory condition of the esophagus. Esophagitis forms a "background disease," so that cells of the esophagus are induced to repair cellular damage and to proliferate. Thus, the rate of synthesis of DNA is accelerated, which can lead to an increased rate of gene mutation, since DNA is more vulnerable to mutation during gene replication. Esophagitis can progress to form precancerous lesions, under the influence of the same fungal toxins that induced

esophagitis in the first place. These lesions can contain foci of initiated cells waiting to be promoted, so that continuous exposure to promoting agents will then lead to cancer development. The most likely promoting agents are either fungal toxins in the food and water, or infection with an esophageal fungus that causes fungal toxins to be produced within the cells themselves. Esophageal carcinogenesis is thus caused by complicated interactions among multiple factors, and cells proceed through multiple stages before they form a cancerous lesion.

CHAPTER 18

Pancreatic Cancer

OCCURRENCE

Carcinoma of the pancreas is currently the fourth most common cause of cancer death in the United States, after cancers of the lung, colon, and breast. Pancreatic cancer accounts for 3% of all new cancer cases each year and 10% of all cancers of the digestive tract. Over 28,000 new cases were diagnosed in the United States in 1990. Carcinoma of the pancreas is more common in men than women, with men half again as likely to contract the disease. Pancreatic cancer is rare before the age of 30, but disease incidence rises steadily with age thereafter. Incidence of pancreatic cancer has increased roughly fourfold over the last 60 years, for reasons unknown.

More than 95% of all pancreatic tumors are adenocarcinomas, with squamous cell carcinomas, islet cell carcinomas, and cystadenocarcinomas comprising the remaining 5% of cases. Because of their overwhelming frequency, only pancreatic adenocarcinomas will be discussed here. In pancreatic adenocarcinoma, cancer cells form glandlike masses arising from epithelial cells of the pancreatic ducts. There is a higher incidence of disease in the head of the pancreas, that portion of the organ closest to the outlet into the duodenum (first part of the small intestine). The head of the pancreas is involved in 65% of pancreatic cancer cases, the body and the tail of the pancreas are involved in 30% of cases, and the tail alone is involved in only 5% of cases.

At the time of diagnosis, tumor is confined to the pancreas in only 15% of patients, and pancreatic cancer tends to be both locally invasive and widely metastatic. About 25% of patients show local invasion of the tumor at diagnosis, while 60% of patients already have metastatic disease. At diagnosis, more than 50% of all patients already have metastases to the liver, more than 33% have invasion of the duodenum, and more than 25% have diffuse seeding of tumor into the peritoneal membranes lining the abdominal cavity. Metastases

to bone and lung are also relatively common. The tendency of pancreatic tumors to be invasive and metastatic may be related to the fact that the pancreas contains relatively little connective tissue, so the organ has a weak, fleshy consistency that offers little resistance to the spread of tumor. Furthermore, the pancreas lacks a capsule, the sheathlike membrane that surrounds organs such as the kidney.

RISK FACTORS

No proven risk factors are known for pancreatic cancer.¹⁶¹ There is apparently no correlation between cancer of the pancreas and acute or chronic pancreatitis, a relatively common inflammation of the pancreas. There does, however, seem to be an increased incidence of pancreatic cancer in patients with gallstones or cirrhosis of the liver.

The incidence of pancreatic cancer is more than twofold higher in smokers than nonsmokers, and about twofold higher in patients with diabetes mellitus. Analysis of the medical charts of pancreatic cancer patients suggests that a diet high in saturated fat is associated with an increased risk of pancreatic cancer. In addition, occupational exposure to certain chemicals (e.g., β -naphthylamine) may increase the risk of carcinoma of the pancreas, and chemists as a group appear to have an elevated risk of pancreatic cancer. Several recent reports have suggested a link between heavy coffee intake and pancreatic cancer, but whether a true causal relationship exists has not been established.

NATURAL HISTORY OF THE DISEASE

The classic symptoms of pancreatic cancer are weight loss, abdominal pain, loss of appetite or food aversion (anorexia), and jaundice. Jaundice is usually associated with disease of the liver or the head of the pancreas, but it can also be caused by certain other disease states such as impaction of a gallstone. Jaundice is a condition in which the skin and whites of the eyes assume a yellowish hue, caused by deposition of the pigment bilirubin from the bloodstream.

Jaundice is essentially a systemic manifestation of local disease affecting the liver, gallbladder, or pancreas, or the ducts interconnecting these organs. Under normal conditions, bilirubin does not accumulate in skin because it is

excreted into the intestines. Bilirubin is a waste product resulting from breakdown of aged red blood cells; hemoglobin within these cells is metabolized to bilirubin in the liver, then excreted into bile. Bile serves several important purposes: it is used to neutralize the acidity of the stomach contents as food moves into the intestines; it is essential for the digestion of fats in the intestine; and it is a way to excrete waste that cannot be excreted in urine. After bile is secreted by the liver, it is stored in the gallbladder, and released into the duodenum as needed after consumption of a fatty meal. Bile is released through the common bile duct, which runs in close proximity to the pancreas. When the common bile duct is obstructed, because of tumor invasion or because of gallstones, bile cannot move into the duodenum. This results in excretion of large amounts of bilirubin into the bloodstream, with consequent deposition in skin.

Jaundice occurs in 80–90% of patients with cancer of the pancreatic head, and in 10–40% of patients with cancer of the body and tail of the pancreas. When it occurs, it is usually progressive and associated with pruritis, or itching of the skin. Either constipation or diarrhea can accompany jaundice. Since jaundice can also be a symptom of liver cancer, care must be taken to differentiate between the two conditions. Pancreatic cancer is often associated with nausea, weakness, fatigue, vomiting, diarrhea, indigestion, and back pain, but all of these can be symptoms of liver cancer as well. Dramatic weight loss is often associated with pancreatic cancer, and there can be a loss of as much as 25 pounds. This weight loss is not fully explained by anorexia and maldigestion, since weight loss by patients with cancer of the tail of the pancreas is often as extensive as weight loss by patients with disease of the pancreatic head. Cancer of the pancreatic tail should not result in obstruction of the common bile duct, so the fact that cancer of the pancreatic tail can result in weight loss suggests that this may be a form of cachexia, or systemic wasting.

Abdominal pain is present in 75–90% of patients with pancreatic cancer. With cancer of the head of the pancreas, pain is likely to be reported in the upper right or upper central portion of the abdomen. With cancer of the body of the pancreas, pain is likely to be reported in the midline, while cancer of the tail of the pancreas is often reported as pain in the upper left quadrant of the abdomen. Abdominal pain may be vague, or it can be a steady, dull, aching pain, often radiating through to the back. In tumors of the body of the pancreas, pain may be severe and unrelenting, although sometimes the pain is modified by changing position. Severe and unrelenting pain can also suggest an advanced cancer, with widespread invasion of the tumor into neighboring organs.

A diagnosis of pancreatic cancer should be suspected in any patient older than 50 who presents with one or more of the following symptoms¹⁶²: jaundice; unexplained weight loss greater than 10% of the normal body weight; upper abdominal pain; unexplained back pain; an attack of pancreatitis without an obvious cause; or sudden onset of diabetes without a predisposing cause such as obesity or family history. For any or all of these conditions, cancers affecting the common bile duct or the duodenum must also be considered, but these cancers are rare.

Physical examination of the patient with pancreatic cancer will often show an enlarged liver, in addition to abdominal tenderness, weight loss, and jaundice. The finding of an enlarged gallbladder (Courvoisier's sign) in a jaundiced patient should immediately suggest obstruction of the common bile duct or one of the other ducts in this area. Such obstruction could be caused by an impacted gallstone, so diagnostic tests for pancreatic cancer must be able to differentiate between malignant obstruction and obstruction resulting from some other cause (Fig. 50). Palpation of the abdomen may also reveal enlargement of the spleen (splenomegaly), caused by invasion or compression of the spleen, or stasis of blood in that organ. The physician may actually use a stethoscope to listen for sounds characteristic of compression of the artery supplying the spleen. In carcinomas of the body or tail of the pancreas, abdominal palpation can actually detect the tumor mass itself in 40–50% of cases.

Tumor grade is established by microscopic examination of biopsy samples taken from the tumor. Grading of pancreatic tumors is very important in determining the feasibility of surgical resection of a tumor, and it is the single best predictor of long-term patient survival. Grading is based on an assessment of the extent of cellular differentiation in the tumor. Cellular features of anaplastic growth, such as a high ratio of nuclear volume to cytoplasmic volume, the presence of darkly stained or oddly shaped nuclei, or the absence of cytoplasmic features associated with normal pancreatic cells, indicate a poor prognosis for the patient. One study found that highly differentiated (Grade I) tumors were unlikely to be invasive of blood vessels, were less likely to surround nerves, and were less infiltrative into normal pancreas.¹⁶³ Grade I tumors can become quite large (up to 4 cm) without necessarily invading surrounding tissue, so prospects for surgical cure of this type of tumor are quite good. Generally, patients with a low-grade malignancy have a good long-term prognosis, irrespective of tumor site or stage, if the tumor can be successfully removed by surgery and if there are no distant metastases.

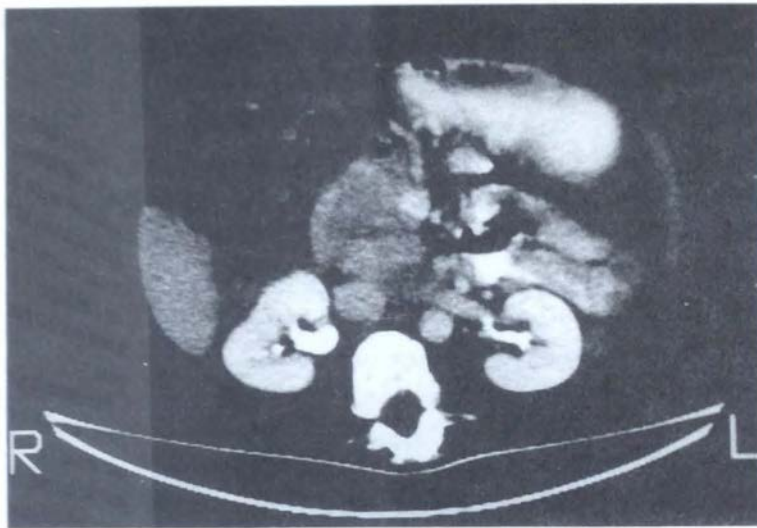


Figure 50. X-ray computed tomogram (CT) of the abdomen of a 40-year-old female patient. She presented with a preliminary diagnosis of gastric ulcer, but endoscopic biopsy of the stomach wall suggested cancer instead. The CT examination of the abdomen was performed to determine whether the stomach was indeed the primary tumor site. This is an image of the lower abdomen in transverse section; a large mass is seen in the center of the image, which has slightly displaced other organs from their normal position. This mass was later determined to be a pancreatic adenocarcinoma that had invaded the stomach wall and the duodenum, so stomach cancer was secondary to the primary cancer in the pancreas. Note the paired bean-shaped kidneys flanking the spinal column at the bottom of the image, the bright mass of the stomach lying obliquely at the upper right of the image, and the liver to the left of the tumor mass. (Photograph courtesy of Dr. Charles Rohrmann and Dr. James A. Nelson, University of Washington School of Medicine.)

Pancreatic resection is the optimal treatment for all eligible patients, and the only treatment that offers a possibility of cure. Unfortunately, relatively few patients are amenable to surgical resection at diagnosis: about 22% of patients with cancer of the head of the pancreas are operable, and only 7% of patients with cancer of the body or tail of the pancreas are operable.¹⁶³ Overall, the proportion of pancreatic adenocarcinomas that are amenable to surgical resection is only about 15 to 20% in most studies. Eligibility for surgery is determined by the extent and invasiveness of the primary tumor, with lymph node involvement being a possible contraindication for surgery, and tumor metastasis probably excluding surgery. Success of surgery is related to degree of tumor differentiation, tumor invasiveness, and the site of the tumor (for Stage II and III tumors). Often none of these parameters can be precisely defined for a

given tumor during surgery, so determining prognosis for the individual pancreatic cancer patient is very difficult.¹⁶³

It should be noted that patients with pancreatic cancer are frequently examined for several months before a definitive diagnosis is reached. Sometimes even exploratory surgery does not provide a definitive diagnosis, since chronic pancreatitis can produce a hard mass in the head of the pancreas indistinguishable by palpation from cancer. Biopsy of such a mass may show no cancer, even if cancer is present, since a small tumor nodule can be surrounded by a large amount of tissue that has the appearance of chronic pancreatitis. Pancreatic cancer is a difficult diagnosis to make until the tumor is far enough along that prospects for patient cure are not good.

BIOLOGY SPECIFIC TO THE TUMOR

The pancreas is an organ that most people are unfamiliar with, until something goes wrong with it. It is an elongated organ extending across the abdomen slightly above the level of the umbilicus. This organ has been called the salivary gland of the abdomen because it secretes pancreatic juice into the intestine. Pancreatic juice is a complex mixture of at least 19 different enzymes that aid in the chemical breakdown of food moving through the intestine, so that nutrients released from the food can be taken up by the intestine and moved into the bloodstream.¹⁶⁴ Pancreatic juice is actually secreted by the exocrine portion of the pancreas; the endocrine portion of the gland secretes hormones associated with cellular absorption of nutrients from the bloodstream. The endocrine function of the pancreas is served by small groups of cells, called pancreatic islets or islets of Langerhans. The islet cells, which comprise only 1–2% of the total volume of the pancreas, are scattered in clumps throughout the body of the pancreas. Islet cells release glucagon and insulin, hormones that regulate the amount of glucose in the bloodstream and the rate at which glucose is taken up by cells. Impaired function of the pancreatic islet cells is responsible for symptoms of diabetes.

If cancer of the pancreas develops, the tumor can interfere with normal organ function, creating fairly serious problems for the patient. If the head of the pancreas is involved, secretion of pancreatic juice may be reduced, so that digestion of food in the intestine is incomplete. If the common bile duct is involved, so that secretion of bile is reduced, this can interfere with digestion of food and excretion of bilirubin. If the endocrine function of the pancreas becomes abnormal, this can have rather dramatic systemic effects, depending

on which hormones are synthesized and released by the tumor cells. If the tumor is extensively invasive or widely disseminated, this can interfere with the function of other organs besides the pancreas.

As is the case with many other tumors, pancreatic cancer is often associated with mutation of a cellular oncogene. In about 90% of all tumors of the exocrine portion of the pancreas, a cellular gene called *K-ras* is mutated.¹⁶⁵ In the remaining 10% of pancreatic tumors, no mutation of *K-ras* is found, but neither is any other cellular oncogene found to be mutated. Thus, pancreatic cancer is very specifically related to mutation of the *K-ras* oncogene. It has been shown that mutation of the *K-ras* gene is sufficient in itself to induce pancreatic cancer in an animal model of human pancreatic cancer. The *ras* gene seems to code for a protein that is probably a growth factor receptor: the normal receptor binds a growth factor, which stimulates the cell to grow, but then the receptor becomes inactivated. Apparently, the mutant growth factor receptor can stimulate pancreatic cells to grow, but the mutation prevents receptor inactivation, so there is a continuous stimulation of growth in cells expressing mutant *ras*. It is noteworthy that the *K-ras* gene mutation is associated with development of tumors in the lung and colon, and mutation of this gene seems to result specifically in adenocarcinoma development.

Mutation of the *ras* gene can be induced by certain environmental carcinogens in experimental models of human cancer. For example, in female rats, exposure to a methyl nitrosourea induces mutation of the *H-ras* gene, with subsequent development of breast tumors. If human *ras* gene mutations are specifically induced by certain carcinogens, then it may be possible to determine which carcinogen is responsible for pancreatic tumor induction.

Recently, ten different human pancreatic cancer cell lines were established in cell culture and grown in a chemically defined culture medium.¹⁶⁶ This is unusual because most human tumor cell lines in culture are grown in an undefined broth containing fetal calf serum, which is a mixture of many different proteins and growth factors. The fact that a cell line will grow in medium with fetal calf serum reveals little about the specific growth requirements of those cells. But when cells can be grown in a completely defined medium, the cell growth requirements are well understood. Human pancreatic cells grown in culture synthesize and release at least 42 different proteins, enzymes, growth factors, and other biologically active substances. Several of these substances may act to enhance the invasiveness and metastatic ability of pancreatic tumor cells in the patient.¹⁶⁶ The finding that cultured pancreatic tumor cells release many different biologically active molecules is consistent with the fact that many pancreatic tumors seem to produce ectopic hormones.

CHAPTER 19

Bladder and Kidney Cancers

OCCURRENCE

The American Cancer Society estimates that bladder cancer afflicted more than 49,000 people in the United States in 1990, making it the fifth most common cancer overall (after lung, colorectal, breast, and prostate). Bladder cancer accounts for roughly 5% of the new cancer cases diagnosed each year in the United States. Another 24,000 cases of kidney cancer were diagnosed in the United States in the same year, making kidney cancer the 12th most common cancer overall. Kidney and bladder cancer together account for about 7% of all new cancer cases in the United States each year.¹⁶⁷

Bladder carcinoma is the most common cancer affecting the urinary tract in both men and women. The bladder is a hollow, saclike organ lined with cells forming what is called transitional epithelium, a type of epithelium unique to the urinary tract. A striking feature of these epithelial cells is that they contain a layer of condensed material at the inner surface of the bladder, which probably serves to protect epithelial cells from the bladder contents. Nevertheless, the lining of the bladder can be exposed to high concentrations of carcinogens in urine. Consequently, bladder carcinomas are often multicentric, meaning that they appear to form several separate tumors, none of which is clearly a primary tumor. Bladder cancer is usually a transitional cell carcinoma (90% of the time), with squamous cell carcinoma of the bladder much less common (8–9% of cases). Bladder cancer afflicts men two to three times more frequently than women, and its incidence is about twice as high for white males as for black males. Bladder cancers usually develop in patients between 50 and 70 years old, and more than 90% of patients are over the age of 50 when diagnosed.

Bladder cancer appears to be a cancer of developed nations and, in the United States, bladder cancer incidence is higher in urban than in rural areas. The highest bladder cancer incidence is found in the industrialized northeastern United States.

The only other urinary tract malignancy that occurs with much frequency is cancer of the kidney. The kidneys are small (5 inches long $\times$ 2 inches wide), bean-shaped organs nestled one on each side of the spinal column, roughly in the small of the back. These organs are responsible for filtration and removal of waste materials from the bloodstream, and production of a concentrated urine to excrete soluble waste. After urine has been produced in the kidney, it moves through the renal pelvis, a funnel-shaped structure that collects urine, and down tubules called ureters. The ureters conduct urine to the bladder, where it is stored until it is voided during urination. Because the kidneys, ureters, and bladder are exposed to any chemicals that are present in the bloodstream, carcinogens have ample opportunity to cause cellular damage in the urinary tract. Cells comprising the urine-producing structures in the kidney, as well as the layer of cells lining the inner surface of the ureters and bladder, may be exposed to highly concentrated carcinogens removed from the blood. Surprisingly, cancer of the ureters is rare, and cancer of the renal pelvis accounts for only 10% of all kidney cancers, so our discussion will be limited to bladder and kidney cancers.

Nearly 85% of all primary kidney cancers are renal cell carcinomas (also called renal adenocarcinomas), with the remaining 15% of kidney cancers comprised of a heterogeneous group of tumors derived from blood vessels, adipose tissue, and smooth muscle.¹⁶⁸ At one time renal cell carcinomas were called hypernephromas, in the mistaken belief that the tumor arose from the adrenal gland, which sits atop the kidney like a stocking cap. This idea has been discarded since histological evidence shows that certain normal cells of the kidney have an appearance similar to renal carcinoma cells, and it is now believed that these cells give rise to renal cancers. Peak age incidence of kidney cancer is between 55 and 60 years old, but there is also a relatively high incidence of kidney cancer in patients younger than 10 years old. Disease incidence is 37–50% higher in men than in women, consistent with the generally higher incidence of urinary tract malignancies in males. Both the incidence and the mortality rate from kidney cancer have increased steadily in recent years, for unknown reasons; incidence increased 27% between 1973 and 1987, while mortality increased 13% in the same period.¹⁶⁷

RISK FACTORS

The major risk factor identified for both bladder and kidney cancer is tobacco, with tobacco use increasing the relative risk of both cancers three- to fourfold. Tobacco smoking is probably responsible for about 50% of all bladder cancers in men living in the Western Hemisphere.¹⁶⁹ A clear relationship has been established between the eventual probability of developing bladder cancer and the dose rate and duration of tobacco use. There is also a correlation between incidence of primary lung cancer and incidence of primary bladder or kidney cancer throughout the world, since these cancers are all induced by tobacco smoke. Greater bladder cancer risk is associated with smoking at an early age, but smoking cessation leads to a dramatic decrease in bladder cancer risk. While the mechanism by which tobacco induces bladder and kidney cancer is unknown, tobacco smoke is known to contain several different chemicals, called arylamines, that are potent bladder carcinogens. Arylamines can chemically bind to DNA, and DNA-arylamine adducts are commonly found in the bladder cells of smokers.

Chemical carcinogenesis is apparently quite important in bladder cancer, and workers in the chemical, dye, printing, plastic, and rubber industries all have an elevated risk of bladder cancer because of occupational exposure to various chemicals. Workers in the dye industry have received particular attention, since several different arylamines used in the manufacture of dyes have been identified as bladder carcinogens. Workers exposed to β -naphthylamine have a relative risk of bladder cancer 87-fold higher than a control group, which accounts for why this chemical is federally regulated and has been under very limited commercial production since 1972.¹⁶⁸ The latent period between chemical exposure and bladder carcinogenesis can be 16 to 35 years. The variability in latency is probably the result of factors such as the particular chemical involved, the intensity of exposure, and the patient age at exposure.

Several nonindustrial chemicals have also been implicated in the causation of bladder cancer.¹⁷⁰ A pain reliever known as phenacetin (no relation to acetaminophen) is occasionally taken to excess by patients, and nearly 5% of patients who abuse phenacetin develop bladder cancer. Phenacetin has also been implicated in cancer of the renal pelvis and kidney. Among patients who contract cancer as a result of phenacetin abuse, the sex ratio is equal, rather than being skewed toward a higher disease incidence in men. Tryptophan, an

essential amino acid, causes bladder tumors in rats when taken in large quantity, and certain by-products of tryptophan metabolism have been found in higher concentration in bladder cancer patients. Artificial sweeteners (saccharin and cyclamates) have been implicated in the initiation or promotion of bladder cancer in rodents, but the evidence linking artificial sweetener use to bladder cancer in humans is weak. Recent evidence suggests that consumption of artificial sweeteners, at the doses and in the manner in which these chemicals are usually used, produces a risk of bladder cancer that is, at worst, very small. A very controversial study reported an association between coffee consumption and bladder cancer in women. No dose–response relationship was established, since bladder cancer risk was the same if one or many cups of coffee were consumed per day, so this finding should be viewed with suspicion. Finally, there is a very clear relationship between exposure to chemotherapeutic agents and subsequent development of bladder cancer. Cyclophosphamide (also called cytoxan) is commonly used for treatment of various malignancies including breast cancer, and this drug commonly produces a bladder inflammation known as cystitis. A number of patients who suffer chemical cystitis as a consequence of treatment with cyclophosphamide subsequently develop bladder carcinoma.

Chronic irritation or infection of the bladder is associated with an elevated risk of bladder cancer. This is implied by the fact that chemically induced cystitis causes an increased risk of bladder cancer, but the relationship between chronic bladder irritation and bladder carcinogenesis is shown more clearly in patients infected with a parasite called a schistosome. Schistosomes are epidemic in various third-world countries and they are spread by contact with infected water. The infective form of the parasite is capable of penetrating unbroken skin and moving through the bloodstream to take up residence in the blood vessels draining the abdominal organs. In the short term, schistosome infection may cause no symptoms, or there may be a fever with flulike symptoms, but in the longer term, schistosome infection can cause liver fibrosis and eventual liver failure. Infestation with this parasite affects over 200 million people world-wide, so this is a very significant world health problem. One species of schistosome (*Schistosoma haematobium*) found in Africa and the Middle East has a tendency to infest the veins of the urinary tract and the wall of the bladder, and this can cause symptoms of bladder infection. Infestation with this parasite has been linked to an increased incidence of bladder cancer in Egypt and Iraq, although typically the resulting cancer is a squamous cell carcinoma rather than a transitional cell carcinoma.

Incidence of bladder cancer is 2- to 3-fold higher in men than women, and this sex difference is consistent for every nation studied throughout the world. It is possible that the higher cancer incidence in men is related simply to the fact that men are more likely to be smokers, or are more likely to suffer occupational exposure to chemical carcinogens. There has been a rather abrupt increase in the number of female bladder cancer patients since 1920, which may be explained by the increased number of female smokers. Alternatively, anatomic or hormonal differences may contribute to the increased incidence of bladder cancer in men. Racial factors also seem to be involved in bladder cancer incidence. Typically black men have a 50% lower incidence of bladder cancer than white men, and black women have an incidence more than 30% lower than white women. The Maori tribe of New Zealand has an incidence of bladder cancer about 20-fold lower than that for white American males.¹⁷¹

Kidney cancer incidence is about 50% higher in urban than rural areas, but there are no racial differences in kidney cancer incidence. Although tobacco is associated with kidney cancer, chemical exposure does not appear to be an important causative factor in kidney cancer. The only known chemical causing renal cell carcinoma is cadmium, which is used in electroplating, welding, and battery manufacture. There is only one hereditary condition known that increases the risk of kidney cancer. An unusual syndrome known as von Hippel–Lindau syndrome, in which patients suffer malformations of blood vessels in the retina and cerebellum, is associated with a higher risk of renal cell carcinoma. The vascular malformations diagnostic of von Hippel–Lindau syndrome begin as benign proliferations of the endothelial cells that form capillary walls. This syndrome is transmitted as a dominant trait, and the clinical symptoms include progressive loss of muscular coordination, headache, accumulation of fluid in the retina, and progressive blindness. Kidney cancer is often the cause of death in patients with this syndrome, and one von Hippel–Lindau family had 17 members afflicted with renal cell carcinoma.¹⁷² Kidney cancer associated with von Hippel–Lindau syndrome is commonly multifocal and often affects both kidneys.

Obesity and a history of myocardial infarction are found more frequently among patients with renal cancer than in a control population, and there is conflicting evidence as to whether diabetes is a risk factor for renal cell carcinoma. Dietary factors, particularly a high fat and cholesterol intake, have been suggested as a potential risk factor for kidney cancer, but this is really rather speculative. Patients with end-stage renal disease who are undergoing

chronic dialysis may develop renal cystic disease and associated renal carcinomas.¹⁷³

NATURAL HISTORY OF THE DISEASE

Cancer of the bladder is usually revealed by the presence of blood in the urine, a condition called hematuria. Nearly 80% of bladder cancer patients experience hematuria, which is often painless, but may be painful if associated with a superimposed bladder infection. Hematuria may be initial, terminal, or total, meaning that blood may be present at the beginning or end of the urine stream, or it may be present during the whole evacuation. If hematuria is noted only initially, this implies that the lesion is close to the outlet from the bladder, while if hematuria is terminal it implies that the lesion is small and bleeds only during contraction of the bladder wall. If hematuria is total it means that blood has been uniformly mixed with urine before evacuation, implying that the lesion may be rather large. Despite the alarming nature of this symptom, the majority of cases of hematuria are not caused by a malignancy.

Bladder irritability, with frequency, difficulty or pain on urination, is present in 30% of patients with bladder cancer.¹⁷⁰ The presence of pus in the urine (pyuria) can also be indicative of a bladder tumor. The presence of sterile urine (indicating no bacterial infection) together with symptoms of bladder irritation or hematuria strongly indicates a need for cancer screening. However, some patients have no clinical symptoms, and diagnosis is often established during evaluation of an abnormal laboratory finding such as occult (inapparent) hematuria. Pain is usually not present with a bladder cancer until the tumor is fairly extensive, unless there is a secondary bladder infection. If the tumor infiltrates the wall of the bladder, there may be severe bladder spasms with pain in the area above the pubic bone. Onset of flank pain is usually delayed until the urinary outflow from the bladder is nearly obstructed.

Renal cell carcinoma is often difficult to diagnose because the symptoms tend to be general, rather than specific to the urinary tract. For this reason, kidney tumors are typically large when discovered, forming spherical masses 1 to 6 inches in diameter (Fig. 51). The classical symptoms of kidney cancer, including blood in the urine, flank pain, and a palpable abdominal mass, are present in only 7% of kidney cancer patients. About 47% of patients present to the physician with hematuria, making this the most common symptom.¹⁷⁴ Hematuria is usually painless, often intermittent, and may be nearly impercept-

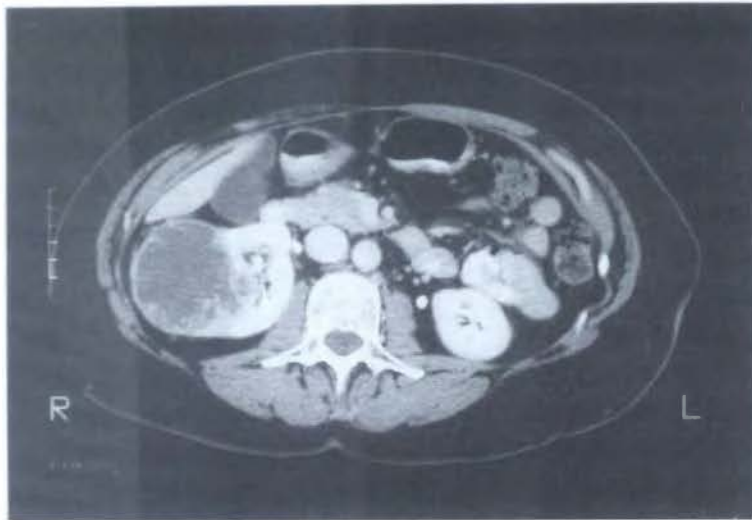


Figure 51. Computed tomogram of a 59-year-old female patient who presented with symptoms consistent with a kidney tumor. The patient's lower abdomen shows a large mass that has replaced much of the normal tissue in the right kidney (to the left in the image). The tumor forms a slightly darkened sphere more than 6 cm in diameter that is covered with a thin rind of normal kidney tissue. Pathological examination of the tumor after radical nephrectomy revealed that the tumor was a clear cell renal cancer entirely contained within the kidney capsule and showing no sign of invasion of other structures. Note the normal kidney lying to the right of the spinal column toward the bottom of the image, and the intestines viewed in transverse section to the upper right. (Photograph courtesy of Dr. William H. Bush and Dr. James A. Nelson, University of Washington School of Medicine.)

ible. Unfortunately, a kidney tumor must achieve a fairly large size before hematuria becomes obvious, and intermittent hematuria may be ignored by the patient for several years. Common local signs and symptoms of renal cell carcinoma include flank pain, which is present in 37% of patients, and an abdominal mass, which is present in 34% of patients. However, in about 18% of patients there are no genitourinary symptoms at all,¹⁷⁴ and renal cancer diagnosis depends on an accidental finding during a physical exam, or on recognition of systemic symptoms of disease by an astute physician.

The systemic symptoms of renal cell carcinoma include anemia, fatigability, cachexia, and fever of unknown origin. Anemia is found in 41% of patients, while cachexia and fatigue are found in 35% of patients, and fever of unknown origin is present in 17% of patients.¹⁷⁴ The origin of these systemic symptoms is unknown, but may be related to production of hormones or hormonelike substances by tumor cells. There is clear evidence that renal

carcinoma cells can secrete certain hormonelike substances, and that these substances can cause syndromes that seem unrelated to the presence of a kidney tumor.

There are several different types of hormonal substances secreted by renal cell carcinomas. For example, the normal kidney secretes a growth factor called erythropoietin, which stimulates formation of red blood cells. Renal tumor cells can secrete abnormally large quantities of this protein, which causes an increase in the number of circulating red blood cells (erythrocytosis) in about 5% of kidney cancer patients. Elevated levels of erythropoietin have been found in the blood in up to 63% of kidney cancer patients.¹⁷⁴ Other hormones normally secreted by the kidney, including renin and various prostaglandins, may also be released in elevated quantities by renal carcinoma cells. Furthermore, renal carcinoma cells can secrete ectopic hormones that are totally inappropriate to the kidney. For example, some renal cell carcinomas secrete parathyroid hormone (normally secreted by the parathyroid gland), prolactin and gonadotropin (normally secreted by the pituitary gland), or glucocorticoids (normally secreted by the adrenal gland).

Ectopic hormone production by renal cell carcinoma can be associated with various complex systemic manifestations of disease, called paraneoplastic syndromes. Ectopic production of parathyroid hormone can lead to hypercalcemia, which is an abnormally elevated level of calcium in the blood caused by release of calcium from bone. Secretion of ectopic prolactin by renal cancer cells causes galactorrhea, in which there is incontinence of milk secretion by the female breast. Inappropriate secretion of gonadotropins by renal cell carcinoma can cause feminization of male patients or masculinization of female patients. Ectopic secretion of glucocorticoids can cause Cushing's syndrome, an odd combination of symptoms in which there is obesity, facial swelling, hypertension, acne, carbohydrate intolerance, and various psychiatric disturbances.

Renal cell carcinoma grows initially by expansion, with the tumor often surrounded by a shell of normal kidney tissue. Eventually, the shell surrounding the tumor is breached and tumor can invade into the fat surrounding the kidney, the renal vein or inferior vena cava, or the adrenal gland atop the kidney. Invasion of the renal vein can cause painful swelling of the testicles, while invasion into the inferior vena cava can cause swelling of the legs; carcinoma cells can even form a solid column of cells within the vena cava. Continued tumor growth can eventually lead to invasion of the muscles surrounding the spine, the spine itself, or the pancreas, colon, or liver. About 10% of patients

with renal cell carcinoma are diagnosed primarily because of metastatic disease, and about 25% of patients have metastases at diagnosis.¹⁷⁴ Common sites of distant metastasis include the lungs, membranes of the thoracic cavity, bone, central nervous system, thyroid, and liver. The lungs are most frequently involved, as nearly 55% of renal cancer patients eventually show lung metastases. Curiously, renal cell carcinoma is the cancer most likely to metastasize to the ear, nose, and throat region, for reasons unknown. Renal cell carcinoma also has a tendency to recur as metastatic disease long after apparently curative therapy. The longest reported interval between surgery for kidney cancer and death from metastatic disease is 50 years.

BIOLOGY SPECIFIC TO THE TUMOR

Cancer of the bladder can present in any of a wide range of forms. *In situ* tumors are tumors that are confined to the surface cells of the bladder wall and that have not yet become invasive. These tumors are often multiple, may co-occur with other types of bladder cancer, and more than half eventually become invasive. Papillary tumors are knoblike tumors attached to the bladder wall by a stalk of tissue, which are often well differentiated. Solid tumors in the bladder wall are likely to be anaplastic and invasive. Anaplastic tumors usually have a flat morphology, cover a large area of the bladder wall, invade the wall deeply, and often have necrotic regions visible at the surface of the tumor. However, even large anaplastic tumors may cause relatively few symptoms. In fact, small tumors that happen to obstruct urinary outflow are more likely to cause symptoms, and to be diagnosed early, than are large tumors that occur within the hollow volume of the bladder. Bladder cancers are usually operable at diagnosis, but they tend stubbornly to recur after surgery. These tumors cause problems more by local invasion and obstruction of the urinary outflow than by metastasis, although metastasis is certainly possible.

Although bladder cancers are quite common, relatively little is known about their biology. Genetic mutations are frequently involved in human cancer, but until quite recently no such alterations had been identified in bladder cancer. However, a recent study found that the p53 tumor suppressor gene, the gene most frequently mutated in human cancer, is often mutated in bladder cancer as well.¹⁷⁵ Analysis of samples of invasive bladder cancer showed that 61% (11 of 18) of these tumors had mutations of the p53 gene. In 91% of cases a mutation of p53 was combined with a chromosomal deletion, so that cells were

left with only mutant forms of the p53 gene product. It was determined that mutations of p53 could be identified in bladder cells that are normally sloughed off into the urine, which should make it possible to develop a screening test to identify bladder cancer by urine testing.

Renal cell carcinoma is unusual even among cancers in that it is particularly difficult to predict the clinical course of the disease. This may be the result of ectopic hormone production by these tumors, since an associated paraneoplastic syndrome can occasionally lead to rapid patient decline. There is also a surprisingly large range of variation in the histological appearance of renal cell carcinoma. Varying degrees of hemorrhage, necrosis, cyst formation, and calcification are often noted, and the color of the tumor can range from gray or white to yellow or bright orange. The architecture of cells within the tumor can also be quite variable, with some tumors forming structures resembling those in the normal kidney, while other tumors have a very undifferentiated, anaplastic appearance. Local invasion or metastasis to the lymph nodes by kidney tumors is typically asymptomatic, and occasionally a primary tumor will remain asymptomatic until distant metastases have been established. This often means that a tumor is well advanced at diagnosis. Yet in other cases, diagnosis may come early in the disease course if the location of the tumor causes hematuria while the tumor mass is still small.

Recently, evidence has been obtained that renal cell cancer risk increases if there is a mutation affecting a specific unknown tumor suppressor gene.¹⁷⁶ Renal cell cancer is frequently associated with a mutation affecting chromosome 3, and inheritance of a mutation at this locus is seen in von Hippel-Lindau syndrome. Laboratory work with rats has shown that mutation of a specific gene can also predispose rats to develop multiple bilateral renal cancers. This mutation is inherited as a dominant gene, suggesting that renal cell cancer in rats can also result from a mutation affecting a tumor suppressor gene. The rat tumors that result from this mutation share several striking similarities with human renal cell cancers: the microscopic appearance of the tumor is similar; the tumors tend to be bilateral and multifocal; tumor cells overexpress a growth factor called transforming growth factor (TGF- α); and the rats typically develop second primary tumors elsewhere, as do patients with von Hippel-Lindau syndrome. When male rats bearing this mutation are exposed to the kidney carcinogen dimethylnitrosamine, they develop an average of 23.0 kidney tumors per animal, whereas normal rats exposed to this carcinogen develop an average of only 0.3 tumor per animal. Thus, a single mutation affecting a tumor suppressor gene caused a 70-fold increase in

susceptibility to kidney carcinogenesis. Interestingly, the relative cancer risk for male rats bearing this mutation was nearly 5-fold higher than for female rats bearing the same mutation, consistent with the higher kidney cancer incidence in males.¹⁷⁶

Two of the tumors that most commonly affect children are tumors of the genitourinary system. Neuroblastoma is a malignancy of the nervous system that arises most often in the adrenal gland. Although this tumor is frequently highly malignant, it also has a high spontaneous regression rate. Interestingly, neuroblastoma *in situ* seems to be a normal feature of fetal development, since examination of the adrenal glands in 169 aborted fetuses showed that every one of the fetal glands had nests of cells that would be classified as neuroblastoma. Apparently these cells are inhibited from forming tumors during the course of normal fetal development.¹⁷⁷ Another study examined 11 infants who died at less than 3 months of age and in whom neuroblastoma cells were found. In every case the cause of death was unrelated to the presence of tumor cells. In 6 cases, the adrenal glands contained microscopic nests of tumor cells, whereas in the other 5 cases the adrenals were partially or completely replaced with tumor tissue. However, most of the tumors showed fibrosis or necrosis, suggesting that they may have been spontaneously regressing. Generally, the older the neuroblastoma patient at diagnosis, the poorer is the long-term prognosis, implying that regression is less likely if the tumor has long evaded processes that normally cause spontaneous regression.

Wilms' tumor, or nephroblastoma, is a renal tumor that is one of the major malignancies of infancy and early childhood. This tumor is noteworthy because if it often familial, and it is inherited in the same way in which retinoblastoma is inherited. The mutated gene in Wilms' tumor has been mapped to human chromosome 11, and the protein product of this gene is apparently a DNA-binding protein that regulates gene transcription.¹⁷⁸ The Wilms' gene maps to the same general chromosomal location as tumor suppressor genes that are mutated in various other cancers, including cancers of the bladder, breast, lung, and liver. Yet mutation of the Wilms' gene is not actually associated with any of these cancers, suggesting that there are several tumor suppressor genes in the same general region of chromosome 11.

The treatment of choice for kidney cancer is generally surgery to remove the involved organ. Chemotherapy of renal cell carcinoma has generally been disappointing in cases of inoperable cancer. Recently, an advisory panel of the Food and Drug Administration (FDA) recommended approval of a new therapy for inoperable kidney cancer. Therapy involves injection of interleukin-2

(IL-2), a biological response modifier that modulates the human immune system. This molecule is normally released by helper T cells and can stimulate T8 cells to develop into cytotoxic T lymphocytes (CTLs). CTLs can directly attack tumor cells, or can stimulate tumoricidal activity by natural killer (NK) cells. If IL-2 is approved by the FDA, it would be the first drug authorized for treating kidney cancer. IL-2 has been shown to shrink kidney tumors in only 10 to 20% of kidney cancer patients, and it can cause serious side effects including lung infection and cardiovascular problems. However, the side effects can usually be controlled and are, in any case, preferable to untreated progression of renal cell carcinoma.

CHAPTER 20

Colorectal Cancer

OCCURRENCE

Cancer of the colon and rectum is one of the commonest cancers in men and women, accounting for about 15% of all new cancer cases. Approximately 155,000 cases of colorectal cancer were diagnosed in 1990 in the United States, so this cancer is second only to lung cancer in terms of overall incidence. Roughly 20% of the annual cancer mortality is comprised of patients with colorectal cancer, and the survival rate for this cancer has not improved appreciably in the past 40 years. It is one of the relatively few cancers that afflicts both sexes equally in the United States, but in other countries colorectal cancer incidence is usually higher in men. These cancers are rare in persons under 35, and only 6–8% of all cases are diagnosed before the patient is 40. Cancer incidence rises steadily with age from 50 to 80 years, and the mean age at diagnosis is about 67.¹⁷⁹

The colon is the large intestine, which extends from the small intestine to the rectum. The colon is arrayed in the abdominal cavity to form three sides of a square, and it is divided into four portions. The right or ascending colon attaches to the small intestine and proceeds upwards, before taking a right-angle bend and continuing across the abdominal cavity, to form the transverse colon. At the left side of the body, the transverse colon takes another right-angle bend and forms the left or descending colon. The descending colon joins to a looped structure called the sigmoid colon, which in turn attaches to the rectum and anus.

The incidence of cancer differs rather strikingly among the different portions of the colon and rectum. Cancer of the anus accounts for only 1% of all cases of colorectal cancer. More than 50% of all colorectal tumors are located in the rectum, and another 20% of tumors are in the sigmoid colon. Thus, more than 70% of all colorectal tumors are sited where they can easily be detected by

examination with a flexible sigmoidoscope, an optical instrument used by physicians to examine the lumen of the rectum and part of the colon. The descending colon, the next most accessible part of the colon, accounts for 6–7% of colorectal cancer cases. The transverse colon is involved in 6–8% of all cases, while the ascending colon is the site of the remaining 15% of cases. The reason for the high incidence of cancer in the terminal portion of the large bowel is unknown, but it may be related to the fact that feces accumulate in the rectum, so that carcinogens in the feces may be in contact with rectal tissue for relatively long periods of time.

About 95% of all malignant tumors of the large bowel are carcinomas, with the remaining 5% made up of gastrointestinal lymphomas (a cancer of lymph tissue), carcinoids (a malignant, but slow-growing cancer), and various sarcomas (cancers of connective tissue). Considering only the bowel carcinomas, about 95% of these tumors are adenocarcinomas, or cancers derived from the epithelial cells lining the intestine. Adenocarcinomas typically develop a glandular or glandlike appearance, and they can secrete large amounts of mucus. Mucus secretion can occur between contiguous cells and it can be copious, so that mucus secretion may actually force cells apart and aid in the invasion of cancer into the colon wall. Anaplastic or undifferentiated adenocarcinomas tend to lose the glandlike appearance, and cell orientation within the tumor becomes increasingly random. In general, anaplastic adenocarcinomas are more invasive than well-differentiated tumors, and the degree of local invasion is the most important variable for determining patient prognosis.

RISK FACTORS

Although the specific causes of colorectal cancer are unknown, a growing number of studies suggest that diet is very important as a causative agent. Studies of the incidence of colorectal cancer in various ethnic groups with different diets have identified a link between incidence of colorectal cancer and consumption of animal fat. There is also an association between colorectal cancer risk and consumption of fat or cholesterol from dairy products, but this relationship is weaker than the association with animal fat from red meat. Incidence of colon cancer is generally low in developing nations and areas where meat consumption is low, while in Europe and North America, where meat consumption is high, colon cancer incidence is also high. Colorectal cancers are unusual in countries such as Mexico, Poland, Greece, and Egypt

(incidence of 3–6 per 100,000 males), while the incidence is much higher in the United States, Australia, Great Britain, Germany, and Switzerland (incidence of 18–23 per 100,000 males). The highest incidence in the world is found in Scotland and New Zealand (incidence of 24–25 per 100,000 males). People who move from a low-incidence area, such as Japan, to a high-incidence area, such as the United States, tend to acquire the higher cancer incidence rate of the adopted country. However, the increased frequency of cancer is usually only manifested after 20 years or more in the adopted country, suggesting that dietary factors take several decades to produce an effect.

Diets that are high in animal fats also tend to be high in total fat, saturated fat, and even refined sugar, so it has been difficult to separate out these variables to determine which is most important in cancer causation. Furthermore, diets high in animal fat also tend to be low in vegetables, and low vegetable consumption has also been linked to a high incidence of colon cancer. However, it is not known whether the presence of animal fats or the absence of vegetables is more important in colon carcinogenesis. Vegetables in the diet could be important because of their fiber content or because of nutrients present in the vegetables. Recent evidence suggests that certain nutrients in vegetables may have an ability to suppress cancer growth. β -Carotene, vitamin C, and vitamin E have all been shown to have a protective effect for cells, perhaps through their ability to trap harmful oxygen free radicals. Oxygen free radicals can damage cellular DNA, so they have an effect on tissues much like radiation (in fact, radiation damage is probably mediated by oxygen free radicals). Thus, the ability to trap oxidants could clearly be beneficial. Cruciferous vegetables (including broccoli, cabbage, Brussels sprouts, and cauliflower) are high in antioxidants, and they can inhibit the induction of colon cancer in experimental tests.

In rodents, consumption of a diet high in saturated fat induces bowel inflammation and loss of cells from the intestinal lining. There is a compensatory increase in epithelial cell growth rate, to regenerate the normal intestinal lining. The mechanism of chemical carcinogenesis appears to be chemical induction of an increased rate of cell division, so chronic stimulation of colon cell division rate by saturated fat is implicated in dietary carcinogenesis. Additional evidence shows that a diet low in crude fiber is associated with an increased risk of colon cancer. The reason for this association is probably that a high intake of dietary fiber causes food to move rapidly through the bowel, so that dietary carcinogens are in contact with the bowel wall for a shorter period of time. Furthermore, dietary fiber tends to increase the water content of

material in the bowel, so carcinogens may move through the bowel in a more dilute state. Finally, there is some evidence to suggest that there may be an interaction between dietary carcinogens and the bacteria that normally colonize the colon. Diets high in animal fat cause an increase in the amount of cholesterol and bile acids in the intestine, which could affect the bacterial flora of the colon. If growth of anaerobic bacteria (which do not require oxygen) is favored, these bacteria may modify the fats or bile acids to yield carcinogens that were not actually present in the diet.

Adenocarcinomas often develop from adenomas (polyps), which are small stalked growths arising from the wall of the intestine and projecting into the intestinal lumen. Benign adenomas are found at autopsy in about two-thirds of all Americans over age 60, and 80% of these adenomas are found in the rectum or sigmoid colon. The sequence of adenoma-to-carcinoma development has been established by examining pieces of intestinal tissue resected during surgery for colorectal carcinoma. About a third of all such intestinal tissue samples contain one or more adenomas. Of patients having benign adenomas associated with the primary tumor, roughly 7% later develop a second primary tumor in the remaining portion of the colon. This rate of cancer incidence is twice as high as in patients who do not have benign adenomas near the primary tumor, suggesting that adenomas can progress to cancer. Multiple polyps are eight times more likely to be associated with cancer than single polyps, and patients diagnosed with multiple primary tumors of the colon or rectum have associated adenomas about 75% of the time.

Controversy exists as to whether all polyps progress to malignancy over time. Polyps less than 1 cm in size are rarely malignant, but as they grow larger, the risk of malignancy increases steadily. Approximately 1% of polyps smaller than 1 cm in diameter contain foci of malignant cells, but in polyps larger than 2 cm there are malignant cells in 50% of cases.¹⁸⁰ Careful histological examination of adenocarcinoma samples reveals that malignant tumors actually incorporate varying amounts of benign adenomatous tissue in 14% of cases. If the adenocarcinomas are subdivided on the basis of tumor stage, the association between adenomas and adenocarcinomas becomes even clearer. In early stage adenocarcinoma, where the primary tumor is small and there is no invasion into underlying structures, adenoma is associated with carcinoma in 60% of cases. When the tumor progresses and is invasive of surrounding bowel wall, there is adenoma remaining in 20% of cases, while in advanced cancer, with invasion to underlying structures, there is adenoma remaining in only 7% of cases. This suggests that adenoma often progresses to carcinoma, but that tumor progres-

sion is associated with destruction or transformation of the benign adenoma component. It has been estimated that the length of time required for progression from adenoma to carcinoma is never less than 5 years, may average 10 to 15 years, and sometimes does not occur at all in a normal life span.¹⁸¹

A hereditary condition, called familial adenomatous polyposis, is strongly predisposing to colorectal carcinoma and is characterized by the presence of numerous polyps (often 1000 or more) throughout the colon (Fig. 52). In the hereditary condition, polyps are not present at birth, but they begin to appear early in childhood and can cause rectal bleeding or diarrhea. Occasionally sporadic cases of adenomatous polyposis are diagnosed, without a family history of the disease, indicating that this condition can arise from a spontaneous mutation. Each individual polyp is initially benign, but the sheer number of polyps means that transformation from benign to malignant is almost certain to occur in one or more polyps. The probability of colon cancer occurring in affected individuals approaches 100% by the age of 40. The only reliable way for affected individuals to avoid colon cancer is to undergo total colectomy and ileostomy, the surgical excision of the entire colon and rectum, together with establishment of an artificial opening through which intestinal contents are emptied.

There are several additional familial syndromes associated with an increased risk of colorectal cancer. Gardner's syndrome is a rare condition in which multiple polyps of the colon and duodenum (the first part of the small intestine near the junction with the stomach) are associated with benign soft tissue tumors and bone tumors. In this syndrome, colonic polyps tend to develop later in life than in adenomatous polyposis, but the malignant potential of the polyps is the same and colectomy is also recommended for these patients. Patients with Gardner's syndrome tend to develop polyps in the stomach and duodenum as well as the colon, and the risk of duodenal cancer appears to be elevated. Turcot's syndrome is a condition in which familial polyposis is associated with an increased risk of central nervous system malignancies. In Turcot's syndrome, colonic polyps tend to be fewer in number (20–100) and larger (3 cm in diameter) than in adenomatous polyposis, and colon cancer tends to develop sooner (10–30 years old). Peutz–Jegher's syndrome is a condition in which multiple polyps develop throughout the entire gastrointestinal tract, with a preponderance in the small intestine. These polyps are unusual in that they result from abnormal development of the organs, and they can include a mixture of different cell or tissue types. The polyps of Peutz–Jegher's syndrome are found in association with dark melanotic spots on the skin, lips,



Figure 52. X ray of a 24-year-old asymptomatic female patient with a strong family history of familial adenomatous polyposis who was examined for the presence of colon polyps. The family history of this patient showed a remarkably consistent picture of polyposis and carcinoma: her paternal grandmother and her father had had polyposis and died of colon cancer, 9 of 13 of her father's siblings had polyposis with carcinoma, and her twin sister was recently diagnosed with polyposis. A barium enema was given shortly before X-ray examination, and numerous polyps are visible as dark spots in the descending colon (to the right). Polyps show as holes in the image because the contrast agent coats the wall of the colon but is too viscous to coat the polyp as well; thus, the hole is the stalk of the polyp and the polyp itself is invisible because it is not coated with contrast agent. These polyps are unusual in that they are solitary and irregularly spaced; often the

polyps of familial polyposis form a carpetlike pattern of contiguous polyps throughout the colon. (Photograph courtesy of Dr. Charles Rohrmann and Dr. James A. Nelson, University of Washington School of Medicine.)

and mucus membranes of the mouth. This syndrome leads to malignancy less frequently than does either familial adenomatous polyposis or Gardner's syndrome, but it is often very difficult to differentiate among the various gastrointestinal polyp syndromes.

In addition to the gastrointestinal polyp syndromes, inflammatory bowel disease is also associated with risk of colon cancer.¹⁸² Inflammatory bowel diseases are chronic inflammations of the bowel whose causation is presently unknown, and the two most important such diseases are ulcerative colitis and Crohn's disease. Ulcerative colitis is characterized by ulceration, blood excretion, and hemorrhage, which is uniform over the length of the colon and often the rectum as well. Ulceration affects only the outermost layer of cells lining the colon, but it can lead to an enhanced division rate of those cells, to regenerate the normal cell lining of the colon. Symptoms of the disease include

bloody diarrhea and abdominal pain, often with fever and weight loss in severe cases. Crohn's disease is similar to ulcerative colitis, but chronic inflammation can affect all layers of the intestinal wall, and the inflammation is usually not uniform over the length of the colon. Segments of the intestine showing inflammation typical of Crohn's disease can alternate with apparently normal segments of intestine. Symptoms of this disease are similar to ulcerative colitis, except that there is a greater likelihood of small bowel involvement and formation of a palpable abdominal mass. Both conditions, when long-standing, are associated with an increased risk of malignancy, and cancer risk increases with disease duration. For ulcerative colitis, the risk of colorectal cancer is 12% after 15 years, 23% after 20 years, and 42% after 24 years.¹⁸³

Human papilloma virus (HPV) has been implicated in the genesis of several cancers, most notably cervical and anal cancers.¹⁸⁴ The most common mode of transmission of HPV is by sexual activity, so sexual promiscuity has been linked to an increased incidence of both cervical and anal cancers. Preliminary evidence suggests that there may be a relationship between the exposure of gay men to HPV during anal intercourse and subsequent development of anal cancer. Gene products produced by HPV include proteins that are able to bind both the p53 gene product and the retinoblastoma gene protein. Thus, HPV can apparently inactivate the two most important human tumor suppressor genes. Research has also shown that infection with the human immunodeficiency virus (HIV), the causative agent of AIDS, greatly increases the risk of developing anal cancer. Interaction between HIV and HPV may promote the course of HIV infection or may make HPV behave more aggressively.

Certain families seem to have a greatly increased risk of colon cancer, with cancer affecting up to 50% or more of the members of these families. However, most cases of colorectal cancer occur in patients with no discernible risk factors, other than age or residence in an area of high cancer incidence. Thus, discussion of risk factors for colorectal cancer is somewhat misleading; while there are established risk factors, the most important risk factors may be unknown.

NATURAL HISTORY OF THE DISEASE

Many of the symptoms of colorectal cancer are not specific for cancer, and can indicate instead peptic ulcer disease, enteritis, diverticulitis, ulcerative colitis, or Crohn's disease. Furthermore, most of the symptoms of colorectal

cancer do not develop until later stages of the disease, so there is a high frequency of delayed diagnosis. The major symptoms of colorectal cancer include blood in the stool, abdominal pain or abdominal mass, weight loss, and changes in bowel habits. Often there is severe constipation, which can be a sign of bowel obstruction, but bloody diarrhea, mucus discharge, or dark-colored tarry stools can be present instead. Tarry stools result from blood loss into the intestinal lumen, with subsequent alteration of the blood by intestinal juices.

The particular symptoms shown by a patient are determined by the size and location of the tumor. Even large lesions of the ascending and transverse colon rarely cause bowel obstruction because of the large diameter of the colon here, and because of the liquidity of the stool at this point in the colon. Tumors of the ascending colon frequently form masses that may be hemorrhagic and can present to the physician as cases of unexplained anemia (reduced red blood cell count), with mild abdominal discomfort or an abdominal mass. Tumors of the descending colon tend to grow around the lumen of the intestine, forming a “napkin-ring” constriction of the bowel, with early signs of bowel obstruction (Fig. 53). Tumors of the sigmoid colon also frequently cause symptoms of bowel obstruction, since the bowel diameter is smaller and the feces are solid when they reach this part of the colon. Cancer of the rectum usually presents as gross blood loss during defecation, and a feeling of incomplete evacuation as the tumor grows and stretches rectal tissues.

Tumors of the ascending and descending colon often form very different types of lesions. Tumors of the ascending colon begin as flat lesions, but as they grow they become polypoid and can form a large, spongy tumor mass. These tumors can become bulky and resemble a cauliflower protruding into the lumen of the intestine, but they rarely cause intestinal obstruction. Tumors of the ascending colon eventually penetrate the intestinal wall and extend into the supportive tissues and lymph nodes around the intestine. Because these tumors do not obstruct the bowel, they often remain clinically silent and produce no distinctive symptoms. Tumors of the descending colon also begin as flat lesions, but they grow around the circumference of the intestine, forming “napkin-ring” lesions, in a process that has been estimated to take at least 1 to 2 years. Deeper layers of the intestinal wall are invaded slowly, but eventually the bowel ulcerates at the point of constriction and the tumor begins to extend into structures surrounding the intestine. Although the growth form of tumors of the ascending and descending colon can be quite different, the tumors look very similar under the microscope.

Patients with ulcerative colitis or Crohn’s disease are difficult to diagnose

Figure 53. X ray of a 58-year-old female patient who presented with intestinal obstruction and other symptoms typical of an advanced colorectal carcinoma. A barium enema was given shortly before the X-ray examination and the barium clearly shows an annular constriction in the middle of the descending colon (to the right). The constriction is the result of multiple irregular polypoid masses, consistent with a diagnosis of adenocarcinoma. Histological analysis of tissue removed at surgery showed this to be an adenocarcinoma with no evidence of secondary tumors or extensive invasion. (Photograph courtesy of Dr. Charles Rohrmann and Dr. James A. Nelson, University of Washington School of Medicine.)



if the inflammatory condition progresses to carcinoma. Many of the early warning signs of colorectal cancer (i.e., rectal bleeding, weight loss, abdominal pain, change in bowel habits) are associated with inflammatory bowel disease as well, so an increase in severity of symptoms is often interpreted as progressive bowel inflammation. This clinical picture is complicated by the fact that patients with inflammatory bowel disease are more prone to cancers in the ascending and transverse portions of the colon, where the tumors cannot be seen by sigmoidoscope. Colorectal cancers that develop in patients with inflammatory bowel disease are often multiple, infiltrating, and highly aggressive. Furthermore, the diagnosis is more difficult to make, even in cases where carcinoma is suspected, because the appearance of the intestinal wall is eroded and very irregular when viewed by sigmoidoscope or other medical imaging technique.

It is not uncommon for colon cancer to invade adjacent bowel, stomach, and abdominal wall without metastasizing to distant areas, so surgical cure of these cancers is possible. However, metastatic spread can occur through both the lymph system and the bloodstream. The likely first sites of metastasis are the lymph nodes surrounding the blood vessels of the abdominal cavity. Late in

the course of the cancer, colorectal cancers tend to metastasize primarily to the liver, but can also metastasize to the lungs and bones.

BIOLOGY SPECIFIC TO THE TUMOR

Familial adenomatous polyposis is associated with an inherited deletion of part of chromosome 5. Sporadic (nonfamilial) cases of adenomatous polyposis do not show the same inherited deletion of part of chromosome 5, but sporadic cases are usually associated with loss of genetic variability at the same chromosomal locus. This suggests that mutation or inactivation of a tumor suppressor gene on chromosome 5 is involved in colorectal carcinogenesis. Two putative tumor suppressor genes, called *APC* (*adenomatous polyposis coli*) and *MCC* (*mutated in colorectal cancer*), have been identified on the involved part of this chromosome. Mutations in *APC* have been seen in cases of both familial and sporadic polyposis, while *MCC* mutations are observed only in sporadic cases. This suggests that although both genes may be involved in development of colorectal carcinoma, only heritable mutations of *APC* actually lead to familial-type polyposis.

Progression of colorectal carcinoma is associated with acquisition of additional mutations affecting other chromosomal sites, so colorectal carcinoma is a clear example of multistage carcinogenesis. After a cancer is initiated by loss of a part of chromosome 5, the cancerous polyp can progress to a more aggressive type of malignancy through rearrangement of chromosome 1 or loss of part of chromosome 17 or 18. Structural rearrangement of chromosome 1 is seen in later-stage colorectal carcinomas, but similar rearrangements are also present in many adenocarcinomas of the ovary, uterus, bladder, and breast, as well as in retinoblastoma and Wilms' tumor (a malignant kidney tumor of young children). Therefore, mutation of a gene on chromosome 1 may be involved in progression of a cancer to a more aggressive or metastatic growth habit. In addition, loss of a part of chromosome 17 was found in 76% of a sample of colon carcinomas, but in only 3% of benign colonic polyps. The gene on chromosome 17 has been identified as the tumor suppressor p53, which is known to suppress growth of a range of different tumors.

Mutation of the p53 gene is the most common genetic change identified thus far in human cancer. The p53 gene is thought to encode a protein that plays a critical role in controlling the cell cycle, since it is known that the p53 protein binds DNA and can regulate gene transcription. Many of the mutations

affecting the p53 gene result in a p53 gene product that is very stable, whereas the normal p53 gene product is unstable.¹⁸⁵ Thus, the p53 gene product can be detected (by monoclonal antibodies) in some tumor cells, while the protein cannot be detected in normal colon cells. Tissue samples from 84 colorectal tumors were examined, to determine whether the p53 gene was aberrantly expressed; it was found that overall, 78% (59 of 76 informative cases) of the colorectal tumors showed abnormal expression of the p53 gene. Of those tumors that showed elevated expression of the p53 gene, about 76% also showed a loss of genetic information affecting chromosome 17. Since this is the chromosomal location of the p53 gene, this indicates that many of the tumor cells were able to express only one (mutated) copy of the p53 gene.

About 70% of colorectal cancers also show a deletion affecting a small part of chromosome 18, so this chromosomal locus may contain a second tumor suppressor gene. The gene on chromosome 18 that is lost in malignant colorectal carcinomas has been christened *DCC* (deleted in colorectal carcinoma). The *DCC* gene codes for a protein that is apparently involved in adhesion of cells to one another. The *DCC* gene is expressed in most normal tissues, including normal colonic cells, but its expression is sharply reduced or absent in colorectal tumors. Alteration of the *DCC* gene may lead to a loss of normal growth regulation, by altering interactions between the tumor cell and surrounding cells or extracellular matrix. It is unclear whether the stepwise chromosomal changes outlined must occur in any particular order, but probably the order of changes is not important; rather it is the progressive loss of a redundant capacity to suppress tumor formation that is critical.

Many of the most malignant colorectal tumors show substantial losses of genetic information in addition to the mutations affecting chromosomes 1, 5, 17, and 18. There is an average loss of about 20% of all genetic information from individual colorectal cancer cells, and some cells suffer a loss of up to half the total genetic information. Tumors with an unusually large loss of genetic information tend to cause an unusually poor patient prognosis. Thus, tumor suppressor genes may be scattered throughout the genome, and inactivation or loss of several of these genes may be required for tumor progression. Malignant tumors differ from benign tumors in that they have progressed farther along a pathway involving stepwise loss of genetic information.

Colorectal tumors are usually curable if they are detected before they become metastatic. Thus, a reliable tumor marker for colorectal cancer would be very useful, especially if such a marker could be used to routinely screen asymptomatic individuals.¹⁸² Hopes were raised in the past because a tumor

marker known as carcinoembryonic antigen (CEA) can be elevated in patients with colorectal carcinoma. CEA is a protein that is normally expressed only during differentiation of fetal intestinal tissue, but CEA is present in the bloodstream of 75% of bowel cancer patients. However, CEA has not proven useful in cancer screening because a number of nonmalignant conditions, including cigarette smoking, chronic pulmonary disease, alcoholic cirrhosis, hepatitis, and ulcerative colitis, also cause elevated CEA. Furthermore, CEA is elevated in 50% of patients with cancer of the pancreas, stomach, lung, or breast. Nevertheless, serial measurements of CEA can be used to detect colorectal cancer recurrence before the tumor produces other clinical signs and symptoms, at a point when there are more treatment options.

Recently, another approach was taken in an effort to develop a screening test for patients with occult colorectal cancer. Any tumor growing in the bowel is expected to shed tumor cells into the bowel lumen, and these cells should eventually be voided from the bowel in the stool. Such cells could indicate the presence of an occult tumor, much as exfoliated cells reveal the presence of cervical cancer in the Pap test. But simply searching for cells shed into the stool would be very insensitive, since such cells would be rare and might even be partially digested. However, if the DNA from these cells could be isolated and screened for a characteristic mutation, this could potentially provide the necessary degree of sensitivity. The *ras* oncogene is mutated in about 50% of colorectal tumors, and 84% of these mutations affect a very specific region of the *K-ras* gene.¹⁸⁶ Therefore, a test for mutations at this specific region of the *ras* gene could enable detection of roughly 40% of all colorectal cancers.

Scientists at the Johns Hopkins University School of Medicine recently developed an extremely sensitive test for *ras* gene mutations, which could be applied to DNA isolated from cells shed into the stool.¹⁸⁶ The test was applied to stool samples collected from nine colorectal cancer patients, whose tumors were known to contain mutations of the *K-ras* gene. In eight of the nine cases, *ras* mutations were detectable in DNA purified from the stool, and the test was so sensitive that even a tumor located in the ascending colon could be detected. Although the sensitivity of this test for colorectal cancer is currently low (i.e., less than 40% sensitivity), test sensitivity could be enhanced by simultaneously screening for other mutations characteristic of colorectal cancer. Such a test could potentially detect colorectal tumors before they become symptomatic or metastatic.

CHAPTER 21

The Gynecologic Cancers

OCCURRENCE

The category of gynecologic cancer includes all cancers of the female genital organs, those complex structures involved primarily in reproduction. While cancer can affect any one of the various structures in the genital tract, the most common sites for cancer are the uterus and the ovary. The uterus, or womb, is a hollow, gourdlike, muscular organ in which a fertilized egg can implant and develop into a fetus. The uterus is lined with a layer of epithelial cells forming the endometrium, with a scattering of tubular, mucus-forming uterine glands that open onto the inner surface of the uterus. The body of the uterus tapers to an elongated neck or cervix, at the end of which is an opening into the vagina. At the opposite end of the uterus from the cervix are the fallopian tubes, which conduct the mature egg from the ovaries to the uterus, and which often serve as the site where fertilization of the egg takes place. The ovary is the organ within which an egg matures, and this organ also has a very significant role in hormone production.

Cancer of the uterus can affect either the uterine endometrium or the uterine cervix. The American Cancer Society estimated that endometrial cancer affected approximately 33,000 women in 1990, or roughly 3% of all new cancer diagnoses. Endometrial cancer is the seventh most common cancer overall, after cancer of the lung, colon and rectum, breast, prostate, bladder, and immune system (lymphoma). The great majority of patients with endometrial cancer are postmenopausal, and the peak incidence of endometrial cancer occurs in the age range from 58 to 60. This is approximately 10 years after the peak incidence of endometrial hyperplasia, a condition that is considered to be a precursor to endometrial cancer. Endometrial cancer is infrequent in young women, with fewer than 4% of cases affecting women under 40 years of age. The incidence of endometrial cancer is generally higher among professional

women and women who delay child bearing, a pattern that is also seen with breast cancer. Histologically, endometrial cancer is almost always a well-differentiated adenocarcinoma, arising from the epithelial cells of the endometrium and forming a glandlike tumor. However, in some cases, endometrial cancer cells resemble flattened or squamous cells, and the resulting tumor is called an adenoacanthoma.

Cancer of the uterine cervix affected about 13,500 patients in 1990, or roughly 1% of all new cancer patients. The relative incidence of cervical cancer has been declining for nearly 40 years, for unknown reasons. During the early years of this century, cervical cancers outnumbered endometrial cancers by a ratio of 3:1, or even 6:1.¹⁸⁷ This ratio began to change in the 1950s, and the ratio had reversed by the late 1960s, so that cervical cancers were less common than endometrial cancers. Currently, endometrial cancers outnumber cervical cancers by a ratio of more than 3:1, so cervical cancer has undergone a 9-fold reduction in relative incidence, compared with endometrial cancer. The average age of women at the time of diagnosis of invasive cervical cancer is about 48, and the precursor conditions to cervical cancer affect correspondingly younger patients. Cervical dysplasia occurs at an average age of 35, while carcinoma *in situ* occurs at an average age of 38. Histologically, 95–97% of all cervical cancers are squamous cell carcinomas, arising from the flattened, scaly cells of the cervix. However, the incidence of adenocarcinoma of the cervix may be increasing, relative to squamous cell carcinoma of the cervix. Most tumors arise at the junction of the cervix and endometrium, where the flattened squamous cells of the cervix grade into the thick epithelial cells of the endometrium.

Cancer of the ovary affected about 20,500 women in 1990, or about 2% of all new cancer cases. Ovarian cancer accounts for about 20% of all malignancies of the female genital tract, being more common than cervical cancer, but less common than endometrial cancer. However, ovarian cancer is the leading cause of death among the gynecologic cancers, reflecting the fact that this cancer is considerably more insidious than the uterine cancers. The incidence of ovarian cancer increases with increasing age, with the peak of incidence occurring at 64 years of age. More than 80% of ovarian tumors are found in postmenopausal women, and these tumors are relatively rare before the age of 50. Fewer than 1% of ovarian tumors are diagnosed in women under 30, and typically younger patients are afflicted with a type of ovarian tumor that is relatively rare in older patients. In the United States, the typical ovarian cancer patient is an older Caucasian woman of northern European ancestry. About

90% of ovarian tumors are derived from the epithelial cells that cover the ovary like a skin. However, the ovary contains a diversity of cell types, and there are consequently a diversity of tumors that can affect the ovary.

The various cancers of the female genital organs together account for 24% of all malignancies in women,¹⁸⁸ or about 6% of all new cancer cases. The incidence of cervical cancer declined 36.4% between 1973 and 1987, while the incidence of endometrial cancer declined 26.1% in the same period, for unknown reasons.¹⁸⁹ There has been a correspondingly large decrease in mortality during the same period; a 39.6% decline in mortality was seen for cervical cancer, while a 19.8% decline was seen for endometrial cancer.

RISK FACTORS

The major risk factor for most of the female genital cancers appears to be exposure to the female hormone estrogen. Estrogen has been implicated as causative, or at least permissive, of cancers of the endometrium and ovary, although it is apparently not involved in cervical cancer. The mechanism by which estrogen exposure increases cancer risk is unknown, but estrogen is known to stimulate the rate of cell growth in the uterus, cervix, and ovary. Malignant transformation of cells is often associated with a stimulus that enhances the normal rate of cell growth, because a dividing cell is more at risk of mutating than is a quiescent cell. Cell division increases the risk of genetic errors of all kinds, and chronic stimulation of cell division is clearly implicated in many different human cancers.

Evidence is quite strong that estrogen is a potent risk factor for endometrial cancer. Continuous administration of estrogen can cause hyperplastic growth of endometrial cells. Certain unusual ovarian tumors (granulosa cell tumors and thecomas), which secrete high levels of estrogen, cause women to have a much greater risk of later developing endometrial cancer. Women with a reduced rate of metabolic degradation of estrogen, secondary to liver failure, often develop endometrial cancer. Women who are obese tend to have higher levels of estrogen in the blood, and these women also have an elevated risk of endometrial cancer. The age group most likely to suffer endometrial cancer is at the menopause, a time during which there are frequent menstrual cycles without a corresponding ovulation. This can result in large amounts of estrogen being released into the bloodstream. Finally, a dose-response relationship has been established, showing that the risk of endometrial cancer increases with

increasing duration of estrogen replacement therapy, or an increasing dose of estrogen.¹⁹⁰

Endometrial cancer is caused by cumulative exposure to estrogen, especially in the absence of opposition by progesterone. Endometrial cells divide in response to estrogen, but the simultaneous presence of progesterone can reduce or even eliminate cell division.¹⁸⁹ Therefore, events that stimulate estrogen release, especially when unopposed by progesterone, increase the risk of endometrial cancer, whereas events that decrease unopposed estrogen exposure also decrease risk. The use of oral contraceptives, which involve daily doses of estrogen and progesterone together for 21 days, followed by 7 days with no treatment, reduces endometrial cancer risk. This is because the endometrium is exposed to unopposed estrogen only during the 7 days when no contraceptives are taken, and blood levels of estrogen are naturally low during this time because of the menstrual cycle. In women who use oral contraceptives for at least 5 years, endometrial cancer risk is reduced 55% relative to that of women who do not use oral contraceptives.¹⁸⁹ Childbearing decreases cancer risk because the high estrogen levels during pregnancy are consistently opposed by very high levels of progesterone. Obesity increases endometrial cancer risk in premenopausal women because obesity is associated with frequent absence of ovulation (anovulation) and a general progesterone deficiency. During anovulation, estrogen levels remain sufficiently high to cause maximal stimulation of endometrial cells. Interestingly, although both endometrial and cervical cancers are uterine cancers, the risk of cervical cancer does not appear to be affected by estrogen exposure.

Hormone exposure does play a key role in ovarian cancer risk. Ovarian cancer incidence is related to ovulation, which is induced by a complex interplay of various hormones, including estrogen.¹⁸⁹ Ovarian cancer usually develops from epithelial cells covering the ovary, and the primary stimulus for division of these cells is ovulation. After each ovulation, epithelial cells replicate to cover the exposed ovarian surface. Factors that prevent ovulation, such as pregnancy or use of oral contraceptives, protect against development of ovarian cancer, with the degree of protection conferred related to the total time of anovulation. However, the protective effect of pregnancy appears to be greater than can be explained on the basis of nonovulation alone. In women who have used oral contraceptives for at least 5 years, the risk of ovarian cancer is reduced 40% relative to that of nonusers.¹⁸⁹

The idea that estrogen exposure is an important risk factor for develop-

ment of both endometrial and ovarian cancer explains a number of seemingly disparate risk factors for these cancers. For example, an elevated risk of endometrial cancer is associated with late menopause, infertility related to nonovulation, menstrual irregularity, or absence of the menstrual cycle (amenorrhea), while childbearing and oral contraceptive use both reduce endometrial cancer risk. An elevated risk of ovarian cancer is found in women who ovulate for more than 40 years, who have never given birth, who first give birth after age 30, or who have a late menopause. All of these separate associations are explained if unopposed estrogen exposure itself is carcinogenic. Conversely, vigorous exercise, lean body mass, and cigarette smoking all lower blood estrogen levels, and all protect against endometrial cancer.

Anything that increases blood levels of estrogen thus acts as a risk factor for endometrial and ovarian cancer. A marked increase in endometrial cancer risk is associated with increasing duration of estrogen replacement therapy after menopause; there is approximately a threefold increase in cancer risk for every 5 years of estrogen replacement treatment, compared with women who do not use estrogen.¹⁸⁹ Similarly, obesity or the consumption of a diet high in calories is associated with an elevated risk of both endometrial and ovarian cancer. High caloric intake in childhood causes early puberty, so that the cumulative lifetime exposure to estrogen is greater. Obese women have an increased risk of cancer because fat cells appear to be capable of synthesizing estrogen, and blood estrogen levels are higher in obese women than in nonobese women. Women who are 30–50 pounds overweight have a threefold higher risk of endometrial cancer, while women who are 50 or more pounds overweight have a ninefold higher cancer risk.¹⁸⁷ The oft-noted association between endometrial cancer and metabolic disorders such as hypertension and diabetes is probably based on the common causal factor of obesity, since obesity is indirectly causative of hypertension and diabetes. The relative risk of endometrial cancer is nearly threefold higher among patients with diabetes, while roughly 50% of endometrial cancer patients are also hypertensive.

While estrogen exposure is the most significant risk factor for both endometrial and ovarian cancer, explaining perhaps 50–60% of all endometrial cancers, family history is also a very important determinant of relative risk of these cancers. The risk of endometrial and ovarian cancers is elevated in women with a family history of gynecologic cancers, especially if the affected relative is a first-degree relation (i.e., grandmother, mother, sister, aunt, or daughter). A positive family history of endometrial cancer is found in 12–28% of endome-

trial cancer patients, suggesting that there can be an inherited tendency to this cancer. Similarly, the relative risk of ovarian cancer is elevated 20-fold in women with an affected sister or mother. The relative risk of ovarian cancer is also elevated in patients with cancer of the endometrium, breast, or colon. However, no genetic markers of cancer susceptibility have been identified, so predictive tests for cancer susceptibility are not available.

Ovarian cancer can also apparently be caused by environmental factors. Therapeutic irradiation of the ovaries at an early age strikingly increases the risk of ovarian cancer 15 to 20 years later. Ovarian cancer is more common among women in industrialized countries, suggesting environmental pollution as a potential causative agent. Ovarian cancer incidence among immigrants to the West is typically higher than in the country of origin, and the cancer rate among immigrants is comparable to the cancer rate prevalent in the adopted country. Exposure to asbestos has been implicated as a cause of ovarian cancer, and an increased risk of ovarian cancer is also associated with the use of talc for genital hygiene. This raises the possibility that foreign material entering the vagina may ascend the genital tract to the ovaries and induce malignant transformation. This idea is not so farfetched when one considers that the fallopian tubes are lined with cells equipped with cilia, presumably to facilitate the passage of sperm up the genital tract.

Viruses such as the mumps virus can cause premature ovarian failure, which could explain a curious association between childhood mumps and ovarian cancer.¹⁹⁰ Women with ovarian cancer less frequently recall having had childhood mumps, although their mumps antibody levels are comparable to the normal population. Mumps is caused by a virus that normally causes inflammation and painful swelling of the parotid gland (near the ear), but this same virus can also cause “silent” or asymptomatic infections of the ovary, testes, or pancreas. It is possible that women with ovarian cancer had “silent” mumps infection of the ovaries, rather than symptomatic infection of the parotid gland.

The most significant risk factors for cervical cancer are all related to sexual habits, and cervical cancer may actually be caused by a sexually transmitted virus. Human papilloma virus (HPV) is a virus that infects the anal and genital tracts, and more than 60 different types of these viruses have been isolated and identified.¹⁹¹ Many HPVs are known to cause venereal warts and other similar benign cellular proliferations. While it has not been proved that HPV causes cervical cancer, the evidence is strong: HPV viral DNA is found in

nearly 90% of all cervical cancers; most of the virus-positive tumors contain viral DNA that is actually integrated into the cellular genome; virtually all virus-associated cervical tumors express specific viral genes; and human cells in cell culture become dysplastic after infection with HPV.¹⁹¹ If cervical cancer is indeed caused by viral exposure, then development of vaccines against HPV is of great potential importance.

Life-style factors that minimize exposure to HPV also minimize the risk of cervical cancer. Thus, barrier methods of contraception (e.g., a diaphragm or condom) reduce cancer risk, while early coitus, multiple sexual partners, or sex with a "high-risk" male all increase the risk of cervical cancer.¹⁹⁰ A "high-risk" male is defined as one who has had multiple sexual partners, or who has a venereal disease known as condylomata accuminata, a condition characterized by warty growths on the penis caused by HPV infection. The incidence of cervical cancer rises with the number of pregnancies. Ethnic or religious groups that encourage monogamy have relatively low rates of cervical cancer, and the disease is very rare in virgins, whereas prostitutes have at least a fourfold greater risk of contracting cervical cancer. Thus, while cervical cancer has not been proven to result from infection with HPV, it is nonetheless a venereal cancer. Other potential causative agents for cervical cancer include: trichomonas, a parasitic microorganism that causes vaginitis; cytomegalovirus, a virus that typically infects the salivary gland; and herpes simplex virus, which has been implicated in oral, as well as anogenital, cancers. It is possible that an interaction among several of these agents is required for the induction of an invasive cervical cancer, and it is likely that women with multiple infections are at greatest risk of developing cervical cancer.

Although the evidence is incomplete, chemical carcinogenesis may be significant in the induction of cervical cancer.¹⁹⁰ At the turn of the century it was a relatively common practice to douche the vagina with coal-tar substances, and this proved to be a potent risk factor for cervical cancer. More recently, it has been shown that tobacco use increases the risk of cervical cancer. Nicotine and other blood-borne components of tobacco smoke are secreted in the cervical mucus of smokers, and this may play a causal role in tobacco-induced cervical carcinogenesis.¹⁹² There is also evidence that vitamin C and folic acid are protective from cervical cancer. This is consistent with the idea that chemical carcinogenesis is important in cervical cancer causation, since both vitamin C and folic acid are "antioxidants," which can potentially protect cells from oxidation and cell damage.

NATURAL HISTORY OF THE DISEASE

Typically, cancers of the female pelvis are rather far advanced before they are diagnosed.¹⁹³ The lack of early detection arises for several reasons, with the most significant reason being that early symptoms of all of these cancers are rather insignificant and mimic the symptoms of menstruation, menopause, or gastrointestinal distress. Even in advanced cases, when obvious symptoms are manifested, these symptoms can be misinterpreted as unpleasant symptoms of menopause, or symptoms resulting from infection with one of the disease-causing microorganisms that affect the female genital tract, or symptoms of gastrointestinal origin. In addition, organs of the female genital tract are rather loosely arrayed over the floor of the abdominal cavity, where there is a good deal of space. This can make it very difficult to detect swelling of an organ such as the ovary, which is normally quite small, until that organ is much larger than normal. This is especially true in an obese patient, or in a patient who has borne many children and whose organs may be somewhat displaced or enlarged. Finally, many patients ignore long-standing symptoms until the symptoms reach a crisis point, at which time a cancer may be well established.

The most common symptom of endometrial cancer is vaginal bleeding, which affects 90% of women with this cancer. Usually the bleeding is slight, and only occasionally is it coupled with minor pain or a feeling of uterine contraction. Vaginal bleeding is a sign of endometrial sloughing as well as endometrial ulceration, so bleeding is characteristic of both menstruation and endometrial cancer. Yet, since endometrial cancer usually afflicts postmenopausal women, it is often possible to rule out menstruation as the cause of vaginal bleeding. However, postmenopausal women receiving estrogen replacement therapy can have occasional bleeding resulting from estrogen. In fact, about 30% of the time, vaginal bleeding in postmenopausal women is actually caused by therapeutic estrogen.¹⁹⁴ Nevertheless, postmenopausal bleeding should always be investigated carefully, since roughly 15% of patients with such bleeding have endometrial cancer. In pre- or perimenopausal women, heavy or prolonged bleeding, or intermenstrual bleeding, should arouse suspicion. The only other symptom that occurs with any frequency in the endometrial cancer patient is pyometra, an accumulation of pus in the uterus, which reveals itself as a vaginal discharge.

Pelvic examination in the early stages of endometrial cancer often fails to reveal the tumor, because uterine enlargement usually occurs only in advanced stages of the disease. As the tumor grows, the shape of the uterus may become

more globular and enlarged, but this is still difficult to distinguish from a uterine fibroid. For this reason, there has been a trend toward routine application of a screening test known as the Pap (Papanicolaou) smear, named after the physician who invented the test. The Pap smear is a cancer screening technique based on microscopic examination of cells swabbed from within the vagina. The test was originally developed as a screen for cervical cancer, for which it is quite sensitive, but about 50% of endometrial adenocarcinomas are also detectable by a Pap smear.¹⁹⁴ The test is less sensitive for endometrial than cervical cancer because tumor cells shed into the uterus are liable to be lysed before they reach the vagina. Nevertheless, any postmenopausal women with endometrial cells apparent on a Pap smear should be carefully examined for endometrial cancer, even if she is otherwise asymptomatic.

Endometrial cancer can form a diffuse tumor spreading over the inner surface of the uterus, or it can form a polyplike mass arising at any point within the uterus. Generally the tumor does not become invasive until it has almost completely filled the cavity of the uterus. The tumor mass is usually soft and easily breaks into small pieces. An endometrial cancer typically spreads along the surface of the uterus and up into the fallopian tubes, although it can also invade into the myometrium, the muscular layer of tissue underlying the endometrium. Occasionally cells will break off the tumor and seed into the vagina, ovary, or abdominal cavity, but this usually does not occur until late in the disease course. Blood-borne distant metastases are not common, and local progress of the disease is blocked by the muscular wall of the uterus, so disease progress is typically slow. Nevertheless, the lymph system can be an important avenue of local tumor dissemination in some patients; for patients with early stage endometrial cancer, lymph node involvement is noted about 13% of the time.¹⁸⁷

The diagnosis of cervical cancer is more difficult than that of endometrial cancer, and the only significant symptom is vaginal bleeding. Since cervical cancer typically affects premenopausal women, vaginal bleeding resulting from cancer can potentially be mistaken for normal menstrual bleeding. Moreover, bleeding only occurs when the tumor ulcerates, and some cervical tumors can be deeply invasive or locally disseminated without causing any ulceration or bleeding. Patients with carcinoma *in situ* are unlikely to have vaginal bleeding, and the only symptom of early stage cancer may be contact staining after coitus, douching, or placing a diaphragm. Even in patients with advanced cervical cancer there may be relatively little bleeding: one study found that only 30% of patients with extensively invasive cancer had experi-

anced bleeding, and bleeding had been present for less than 2 months.¹⁹⁵ Abnormal bleeding is the only symptom of cervical cancer that is likely to bring diagnosis early enough for the cancer to be still curable. Yet cervical cancer is considered to be a preventable cancer, because it has a long precancerous phase, because a sensitive screening test is available, and because treatment of reinvasive lesions is effective.¹⁹⁶

As cervical cancer progresses, the tumor infiltrates into adjacent structures and begins to destroy them (Fig. 54), and the severity of symptoms depends on the degree of tissue destruction. Direct invasion can affect the vagina, the body of the uterus, and the connective tissue of the pelvic floor. A malodorous discharge from the vagina can occur if there is a bacterial infection, secondary to tissue destruction. Pain is usually not noted until the tumor invades the nerves of the pelvic wall, and pain is likely to be perceived as a persistent aching pain of the lower abdomen or back. Patients with advanced



Figure 54. Magnetic resonance image (MRI) of a 54-year-old female patient who was examined for the possible presence of a cervical tumor. This image is somewhat different from the other images in this book because the patient was imaged while lying on her side. The patient is facing left, with the right leg visible at the bottom of the image. The spinal column is to the right in the image, curving down and ending near the rectum. The adjacent structure to the left is the vagina, visible as a thick-walled structure with a central canal angling toward the top left of the image. At the top of the vagina a very large cervical tumor has displaced other structures and fills much of the central portion of the abdomen. The tumor itself is mottled in appearance, appears to be necrotic (upper right of the mass), and may be invading structures to the left of the tumor mass. The uterus has been displaced upwards and shows as a very bright oval mass, suggesting that it is filled with an accumulation of fluid because the tumor is

obstructing drainage of the organ. (Photograph courtesy of Dr. James A. Nelson, University of Washington School of Medicine.)

disease may suffer kidney failure, secondary to the obstruction of kidney drainage by the growing tumor mass. Swelling of the legs indicates lymphatic obstruction, which typically only occurs in advanced disease. Formation of a fistula, an abnormal passage from the cervix through to the intestine or bladder, is an indication of very advanced disease. Cachexia, the wasting syndrome associated with cancer, is also present only in patients with a very advanced cancer. In advanced-stage cervical cancer patients, metastasis to regional lymph nodes is common (44–55% of patients), but metastasis to more distant nodes is less common (30–40% of patients).¹⁹⁶ Distant blood-borne metastases are rare, and in the overwhelming majority of cases, cervical cancer is confined to the pelvic floor even in very advanced cases.¹⁸⁸

Often a diagnosis of cervical cancer is made on the basis of a routine Pap smear, which can detect *in situ* cancer in an apparently normal cervix before there is any visible sign of a lesion. For this reason, women have been urged by the American Cancer Society to get regular Pap smears after the age of about 40. The routine application of Pap smear screening has revealed some details of the natural history of cervical cancer. Pap smears typically detect carcinoma *in situ* when there is an increased rate of cell proliferation and some cellular atypia, but there is not yet invasion of tumor cells into the cervix. Age at onset of carcinoma *in situ* averages about 10 years less than age at onset of invasive carcinoma, implying that cervical cancer is a very slow-growing tumor. It has been assumed that progression from dysplasia, to carcinoma *in situ*, to invasive carcinoma would occur in every case without treatment, but this may not be true. Malignancy can arise without a preexisting dysplasia, and some dysplasias may not progress.

Probably the most difficult diagnosis among the gynecologic cancers is ovarian cancer. There is no screening test available for this cancer, and often the woman with early ovarian cancer has no symptoms at all. If symptoms are present, they can be mild and characteristic of gastrointestinal distress. Insidious signs of early ovarian cancer include: vague abdominal discomfort; indigestion (dyspepsia); flatulence; bloating; urinary frequency; and constipation.¹⁹⁷ These symptoms are obviously not specific for cancer and may be tolerated for months before the help of a physician is sought. Symptoms of an advanced ovarian cancer include abdominal distention and pain, presence of a pelvic mass, and vaginal bleeding. With the exception of vaginal bleeding, even these symptoms can be mistaken for a problem that is gastrointestinal rather than genital in nature. The most important sign of ovarian cancer is the presence of a solid, irregular, or fixed pelvic mass on physical examination.

Because diagnosis of this cancer is so difficult, physicians look for the following set of conditions that can suggest ovarian cancer: a patient aged 35 or older, with persistent but unexplained symptoms of gastrointestinal distress and a long history of ovarian malfunction.

Because ovarian cancer is so difficult to detect, it typically reaches a large size before diagnosis and, in more than two-thirds of patients, the cancer has spread to surrounding organs at diagnosis. Ovarian cancer usually spreads by implantation, a type of superficial invasion. Ovarian cancer frequently seeds itself over the surface of adjacent organs without invading deeply into those organs, so that tumor cell growth is confined to the surface of adjacent structures. Tumor extension into adjacent structures can involve the intestine, bladder, and rectum, or the membranes covering these organs. However, tumor dissemination through the lymphatic system or through the bloodstream can also occur. The lymphatic drainage of the abdominal cavity may be obstructed, leading to ascites, a condition in which there is accumulation of a large amount of lymphatic fluid within the abdominal cavity. Spread of ovarian cancer through the bloodstream is usually not seen until late in the disease course, but eventually the lungs, liver, bones, and central nervous system can be sites of metastasis. Hormonal manifestations can also be seen with ovarian cancer, and symptoms can include masculinization of the patient, or rampant oversecretion of estrogen resulting in endometrial hyperplasia.

BIOLOGY SPECIFIC TO THE TUMOR

Endometrial cancer risk is higher in women of upper income levels, which is rather unusual. The only other major cancer that is more common among relatively affluent women is breast cancer, yet endometrial and breast cancer together affect more than one-third of all female cancer patients. In general, there appears to be an inverse relationship in the incidence of endometrial and cervical cancer. In the highly industrialized countries of the West, endometrial cancer is relatively common and cervical cancer rare, whereas in Latin America, cervical cancer is relatively common and endometrial cancer rare.

Recently a great deal of attention has been focused on the molecular biology of human cancer, with particular attention paid to the role of tumor suppressor genes. Just as certain cancers can be caused by activation of an oncogene, other cancers result from inactivation of a tumor suppressor gene. Inactivation of the p53 tumor suppressor gene is thought to be involved in

development of cancers of the colon, breast, lung, and brain. One recent study analyzed the p53 gene in cultured cell lines derived from human endometrial carcinomas. The DNA encoding the p53 protein was sequenced in six human endometrial carcinoma cell lines, and mutations affecting the p53 gene were found in every cell line tested.¹⁹⁸ However, mutations affecting the p53 gene were found in only 13% of endometrial tumors,¹⁹⁹ so mutation or inactivation of p53 is not an obligatory step in the development of endometrial cancer.

Squamous cell carcinoma of the cervix appears to be the end stage of a disease progression that begins with dysplasia, progresses through carcinoma *in situ*, and culminates in invasive disease. The transition from dysplasia to invasive cancer can take from 8 to 20 years, so the process is quite slow. The slow progression of cervical cancer means that Pap screening need be done no more frequently than once every 3 years, after an initial baseline has been established by several Pap smears at about age 20. Studies of large groups of women have demonstrated unequivocally that use of the Pap smear reduces mortality from cervical cancer. In fact, for women who have had Pap smears at least every 3 years, there is a 90% reduction in risk of invasive cervical cancer, compared with women who have not been Pap tested.¹⁹⁰

Generally speaking, there is a correlation between the growth rate of a tumor and the ability of that tumor to induce angiogenesis, or the growth of new blood vessels into the tumor.²⁰⁰ The extremely slow growth of cervical tumors may mean that these tumors are not good at inducing angiogenesis. This is consistent with the fact that the amount of oxygen present (i.e., the oxygen tension) within cervical tumors is usually very low. The oxygen tension of cervical tumors is well below that of normal cervix, and even below the oxygen tension of tumors at other sites in the body. In cervical tumors, the oxygen tension tends to decline as the tumor progresses: normal cervix has an oxygen tension of 36 (mm Hg mean partial pressure of oxygen), while Stage 0 cancer (carcinoma *in situ*) has an oxygen tension of 20, Stage I has an oxygen tension of 13, and Stage 2 has an oxygen tension of 5.²⁰¹ Cervical tumors are thus inadequately oxygenated and there may be large portions of the tumor that are hypoxic. This is extremely important because it has implications for the likely success of tumor treatment.

The success of radiation therapy for cervical cancer depends on the presence of oxygen within the tumor tissue, because radiation kills cells by causing the formation of highly cytotoxic molecules called oxygen free radicals. Since tissue oxygen is the only source of oxygen free radicals, well-oxygenated tissues are about threefold more sensitive to radiation than are

hypoxic tissues. If tumor tissue is poorly oxygenated, this implies that the delivery of oxygen to the tumor is impaired, and that tumor perfusion is inadequate. If oxygen delivery to the tumor is impaired, then probably the delivery of blood-borne chemotherapeutic agents to the tumor will be similarly impaired. Thus, both radiation therapy and chemotherapy are less likely to be successful in cervical tumors, since these tumors are hypoxic to start with. One study showed that the degree of tumor hypoxia did indeed determine the success of radiation therapy in patients with advanced cervical carcinoma.²⁰² It was observed that patients with anemia (reduced oxygen-carrying capacity of the blood) responded poorly to radiation therapy for cervical cancer. Since anemic patients are more likely to have extensive hypoxia within their tumor, correction of that anemia might lead to an improved tumor response to radiation. A prospective study of patients with cervical cancer showed that correction of anemia led to a decreased rate of recurrence of cervical cancer, and an increased tumor cure rate.

Evidence is accumulating that cervical cancer is caused by a sexually transmitted virus.¹⁹¹ HPV infects the anal and genital tracts, and exposure to two particular types of HPV has been linked to a high risk of cervical cancer. The viral DNA codes for two proteins, called E6 and E7, which bind to and inactivate the tumor suppressor genes p53 and RB, respectively.²⁰³ The p53 gene is the single most frequently mutated gene in human cancer, and inactivation of the RB gene has been associated with development of several cancers, including retinoblastoma and cancers of the breast, lung, and colon. Loss of the normal function of both the p53 and RB tumor suppressor genes, either as a consequence of inactivation by E6 and E7, or as a result of mutation, appears to be a common event in human cervical carcinogenesis. In fact, preliminary results suggest that all cervical cancers may result from inactivation, mutation, or loss of the products of the p53 and RB genes.²⁰³

New evidence suggests that mutation or loss of the tumor suppressor gene p53 is also common in ovarian cancer. Tissue samples from 31 ovarian cancers were tested for loss or mutation of the DNA encoding the p53 gene. Loss of the p53 gene was detected in 16 of 20 cases, while mutation of this gene was detected in another 9 of 12 cases, so that overall 75–80% of ovarian cancers show alterations of the p53 gene. Mutations affecting the p53 gene were identified in tumors ranging from early to late stage, suggesting that this mutation occurs early in tumorigenesis.²⁰⁴ Thus, inactivation, mutation, or loss of the p53 gene may play an important role in the development of endometrial, cervical, and ovarian cancer.

Taxol, an extract from the bark of the Pacific yew tree, is now being used as an experimental drug in the treatment of ovarian cancer. Human trials began in 1983 and the drug soon showed promise against ovarian tumors that had been resistant to treatment by any other method. Taxol has also shown promise against cancers of the breast, prostate, and skin. Although the National Cancer Institute has made development of this drug an "emergency priority," in 1991 it was available to fewer than 5% of the patients who could potentially benefit from the drug. The problem has been that the yew tree takes 70 to 100 years to grow to 10 inches in diameter, and it is often difficult to find trees this large. In the past, the yew was considered to be a weed, and logging companies often felled small yews merely to gain better access to more valuable trees. A complete taxol treatment for a single patient requires the amount of taxol that can be extracted from the bark of two or three trees that are 70 to 100 years old. It has also been difficult to extract taxol from yew bark in a process that can be done on an industrial scale. Although recent efforts have enabled chemists to synthesize taxol from chemical precursors, it is still too soon to tell whether this process can be implemented on an industrial scale.

CHAPTER 22

Prostate Cancer

OCCURRENCE

The prostate gland is a walnut-shaped gland, nestled beneath the bladder, which is involved in semen production. Prostate cancer is the most common cancer in men, and the second leading cause of cancer death (after lung cancer) in men over 55 years of age. The American Cancer Society estimated that there were about 106,000 new cases of prostate cancer in 1990, representing roughly 10% of all new cancers. More than 92% of all male genital cancers are cancers of the prostate.²⁰⁵ Other male genital cancers include cancer of the testis (6%) and the penis (2%), with cancer at other sites making up less than 1% of the total.

Over 95% of prostate cancers are adenocarcinomas that arise in the secretory structures of the prostate, which are called acini.²⁰⁶ Only about 20% of prostate cancers are limited to the prostate at the time of diagnosis. Although prostate cancer is rare in men under 55, it has been estimated from autopsy data that 25% of men aged 70 or older have a clinically undetectable early form of prostate carcinoma at the time of death. In contrast, about 3% of men of this age have clinically detectable prostate carcinoma, suggesting that only 12% of the total number of potential patients are actually diagnosed. Several studies have reported an increasing incidence of the disease, but there has been no significant increase in mortality from prostate cancer since 1950. Since incidence has increased without a corresponding increase in mortality, this suggests that detection rate of the less malignant early form of the disease has improved.²⁰⁵

RISK FACTORS

The prevalence of clinically occult (undiagnosed) prostate cancers determined at autopsy is similar throughout all countries and racial groups.²⁰⁷ However, the frequency of clinically diagnosed prostate cancer varies markedly in different parts of the world. In the United States there are 14 deaths per 100,000 men per year from prostate cancer, compared with 22 deaths per 100,000 in Sweden and 2 per 100,000 in Japan. Although the incidence of prostate cancer in Japan is very low, the incidence in second-generation Japanese male immigrants to the United States is similar to that of other men in the United States. This suggests that there may be an unknown environmental factor that predisposes to prostate cancer. The disease is more common among black men than among white men in the United States, but the reason for this difference is also unknown.

The greatest single risk factor for prostate cancer is advanced age. Cancer of the prostate occurs primarily in older men, with an average age at diagnosis of 70. Autopsy studies have shown evidence of prostate cancer in over 40% of men in their ninth decade who died from unrelated causes. Increased incidence of the disease in recent years may reflect the graying of the population in the United States. Thus, as the population continues to increase in average age, it is expected that the incidence of prostate cancer will also increase.

Carcinoma of the prostate occurs more frequently in the sons and siblings of patients who developed prostate cancer. A fourfold increase in the incidence of prostate cancer is seen in the brothers of men who developed prostate cancer by the seventh decade of life.²⁰⁷ For any given man, the more first-degree relatives (e.g., grandfather, father, brother, uncle, or son) diagnosed with the disease, the greater is the risk of prostate carcinoma. Whether this indicates a genetic predisposition to the cancer or simply reflects shared exposure to an environmental carcinogen is unknown.

There is no convincing evidence that a prior history of prostatitis or urethritis predisposes a patient to the development of carcinoma of the prostate. Conflicting evidence is found in the literature as to whether there is connection between sexual activity and prostate cancer: some researchers have reported that sexual hyperactivity is associated with prostate cancer, while others have reported that celibacy is associated with the cancer.²⁰⁷ There is some evidence that a high-fat diet contributes to cancer development, but there appears to be no connection between cigarette smoking or alcohol consumption and development of prostate cancer.

NATURAL HISTORY OF THE DISEASE

Both early and advanced prostate carcinoma may be asymptomatic at diagnosis and diagnosis commonly occurs during a routine rectal examination. This shows the importance of the rectal examination as part of the routine physical examination of all men over about 40 years old. Among newly diagnosed patients, 80% have disease extending beyond the prostate at the time of diagnosis.

In those patients experiencing symptoms resulting from prostate cancer, the most common symptoms are (in descending order): difficulty or pain on urination; difficulty in complete voiding of the bladder; increased urinary frequency; urinary retention; back or hip pain; and blood in the urine. Any man over 40 with difficulty in urination, urinary frequency, or difficulty in voiding should be regarded as a candidate for a thorough prostate examination.²⁰⁸

Manual palpation of the prostate remains the most common means by which a prostate cancer diagnosis is made. Prostate carcinoma occurs most frequently in the posterior part of the prostate, which is easily palpated during a digital rectal examination. Carcinoma is characteristically hard, nodular, and irregular (Fig. 55), but benign prostatic hyperplasia can also lead to formation of firm nodules, so digital examination alone cannot be used to differentiate between hyperplasia and carcinoma. Local invasion of tumor into the seminal vesicles can often be detected by a digital examination, and this finding is sufficient to diagnose carcinoma. Swelling in the scrotum or legs caused by water retention occurs if a tumor is extensive and obstructs lymph drainage of the groin.

The capsule, or covering of the prostate, provides a natural barrier to extension of prostate carcinoma, but this barrier is frequently breached in patients by the time cancer is diagnosed. The only way to definitively determine whether local extension of the tumor has occurred is for the surgeon to inspect the area around the prostate at the time of surgery. Histologic grading of a prostate carcinoma depends on microscopic examination of a sample removed at surgery. Histologic examination of the prostate typically shows a great deal of variability in cellular size, nuclear size and shape, degree of differentiation of cells forming the secretory structures (acini), and the enzyme and mucin content of the acini. Generally, the most poorly differentiated area of the tumor, or that portion of the tumor that is most anaplastic, tends to determine the course of the disease. The Gleason grading method is commonly used to objectively describe the degree of anaplasia in a prostate tumor. In this method,



Figure 55. Magnetic resonance image (MRI) of a 57-year-old male patient who was screened for prostate cancer during a routine physical that included a digital rectal examination. The physician noted an enlarged prostate palpable through the rectal wall and referred the patient for a follow-up examination. The image shown here was obtained by magnetic resonance imaging of the pelvis, with the pelvis viewed across the long axis of the body. The pubic bone is at the top of the image, the muscles of the buttocks are out of frame to either side, and the rectum is at the bottom of the image. The bladder forms a large transverse-lying oval below the pubis, and the prostate appears as a vertical oval beneath the bladder. Note that the prostate appears nearly as large as the bladder; this is misleading because the bulk of the bladder is above the prostate, so any view of the prostate shows only a small portion of the bladder. The prostate appears enlarged and mottled in this image, suggesting malignancy, and biopsy proved this to be an adenocarcinoma. (Photograph courtesy of Dr. Jay Tsuruda and Dr. James C. Nelson, University of Washington School of Medicine.)

ps

The dominant histologic pattern is assigned a number from 1 to 5, with 5 indicating a poorly differentiated, highly anaplastic tumor. Then any other glandular histologic pattern is similarly evaluated, and the two numbers are summed to give a Gleason grade between 2 and 10. Numbers at the upper end of the scale indicate a highly anaplastic, presumably aggressive cancer. The Gleason grading method is surprisingly reproducible among different histologists examining the same sample, and Gleason grade correlates well with disease course.

Tumor staging is accomplished by consideration of the extent of dissemination of the primary tumor. Adenocarcinoma of the prostate spreads beyond

the prostate capsule by three different routes. These three routes are by direct extension into adjacent structures, through the lymph system, and through the bloodstream. Direct extension usually occurs into the seminal vesicles and into the bladder wall. Lymphatic spread of prostate adenocarcinoma must be assessed at the time of surgery, since lymph node involvement is correlated with the size and histologic grade of the tumor. Spread of prostate adenocarcinoma through the bloodstream may produce distant metastases that cannot be detected at surgery. The most common sites to which this tumor metastasizes are (in descending order of frequency): local bony structures (pelvis and lumbar vertebrae), distant bone (thoracic vertebrae and ribs), and the lungs, liver, and adrenal glands.

Approximately 70% of patients with prostate cancer develop metastatic tumors in bone, so the prostate cancer patient is usually carefully screened for bone metastases.²⁰⁹ The pelvis and lower vertebrae are the most frequent site of metastasis, but metastases can also occur in vertebrae of the thorax, as well as in the ribs, skull, and long bones of the legs and arms. Positive bone scans are associated with any metabolic condition that leads to elevated metabolism of bone cells, including metastases to bone, healing fractures, and arthritic or inflamed sites. When a positive bone scan is obtained during an examination for bony metastases, these other types of pathology must be excluded before the site should be treated as a metastasis. Bone scans can be very useful for monitoring tumor progression or assessing response of metastatic disease to treatment.

Surgery to remove the prostate, known as radical prostatectomy, is the treatment of choice for all prostate carcinomas that are not extensively invasive, and it is the only treatment that offers the possibility of complete cure. Radical prostatectomy is a fairly difficult surgery, and patients undergoing the surgery are subject to the same complications as any major pelvic surgery. The incidence of complications such as wound infection, venous thrombosis (blood clots in the veins), and cardiovascular or pulmonary complications is relatively low. However, there are several complications of radical prostatectomy that are unique to the surgery, including urinary incontinence, impotence, rectal injury, and stricture or narrowing of the urinary outlet from the bladder. Urinary incontinence is generally suffered by most patients for several months following surgery. Return of urinary control after radical prostatectomy is a gradual process that may take 3–6 months, and complete control may not be reestablished until 9 months or a year after the operation. Approximately 2% of patients will have significant postoperative incontinence more than a year after

surgery, while an additional 15–20% of patients may have minor to moderate stress incontinence.

Radical prostatectomy at one time resulted in erectile impotence in more than 90% of patients, but this situation has changed with the introduction of a new type of surgery. In the past, radical retropubic prostatectomy was known for the extreme amount of bleeding that occurred during surgery, so that the surgeon often had to work in an area obscured by a pool of blood. However, in 1982, a new surgical technique was developed that does less damage to vessels draining blood from the pubic area, so that surgery can be performed in a “bloodless field.” This means that surgeons can actually see what they are doing, with the result that the surgery is safer, fewer structures are inadvertently damaged, and the nerves responsible for penile erection can be spared. Postoperative potency rates average about 75% in patients who are operated on by surgeons experienced with this new technique, called the Walsh procedure.

BIOLOGY SPECIFIC TO THE TUMOR

Autopsy studies have shown that many men who die of natural causes nevertheless have a clinically undetectable prostate adenocarcinoma. These men were able to live a normal life without any symptoms of cancer, even though there was cancer present. This is only possible because prostate cancer is one of the slowest growing of all malignancies. Even patients diagnosed with a palpable tumor of the prostate can live a normal life and die of unrelated causes. Careful consideration must be given to the extent of treatment advisable for a given patient since, in the short term, the effects of treatment can be worse than the effects of disease. This is especially true in recent years, as there is a trend toward detecting prostate cancer ever earlier in the disease course.

Growth of the normal prostate gland is dependent on the presence of male hormones called androgens. The major androgen circulating in the bloodstream of men is testosterone. About 90% of all androgens are secreted by the testes, with the adrenal glands making up the difference. Because of the hormone dependence of normal prostate tissue, it is logical to attempt to halt progression of prostate cancer by androgen deprivation (Fig. 36). This involves removal of the source of those hormones on which prostate cell growth is dependent. Androgen deprivation can be achieved in one of four ways:

1. Surgical removal of the glands that secrete androgens. This involves removal of both the testicles (castration or orchiectomy) and the adrenal glands (adrenalectomy).
2. Inhibition of the hormones that stimulate androgen production. The pituitary gland secretes two hormones in both men and women, known as follicle-stimulating hormone (FSH) and luteinizing hormone (LH). While these hormones are more familiar in women, they are also present and active in men; FSH stimulates sperm production, while LH stimulates the synthesis and secretion of androgens by the testes. Inhibition of the androgen-stimulating activity of LH in men can be accomplished by hormone therapy or removal of part of the pituitary gland.
3. Direct inhibition of androgen production. A drug called aminoglutethimide has been used to block androgen synthesis by the adrenals and the testes. While this drug can achieve a level of androgen ablation similar to surgery, it is reversible should this treatment fail.
4. Inhibition of androgen binding to the androgen receptors in the tumor. This can be accomplished by administration of drugs such as cyproterone or flutamide.

The most common ways of achieving androgen deprivation in the clinic are castration or estrogen therapy. Since testicular secretion accounts for upwards of 95% of all testosterone production, bilateral orchiectomy can result in a 90–95% decline in androgen levels in the blood. Estrogen therapy, usually using an estrogen analogue called diethylstilbestrol (DES), can have similar effects on plasma androgen levels. If further depletion of plasma androgen levels is required, removal of the adrenal glands can also be done. However, removal of plasma androgens has a major impact on the patient as well as on the tumor. Orchiectomy results in feminization, loss of the secondary sex characteristics such as beard growth and muscle mass, and consequent psychological stresses on the man experiencing these effects.

Androgen-deprivation therapy has been a standard therapy for prostate cancer for many years, and it is still the most common treatment for metastatic carcinoma of the prostate. Several recent prospective clinical studies have compared the survival of patients treated with androgen deprivation and patients who were not androgen-deprived. These studies failed to establish the effectiveness of androgen deprivation in enhancing survival of patients with

early stage prostate cancer. Thus, it seems reasonable to reserve androgen-deprivation therapy until disease progression is evident. Since androgen deprivation can significantly palliate certain symptoms of advanced prostate cancer, some patients may be good candidates for this therapy. In addition, low doses of DES appear to decrease deaths from prostate cancer, even at a dose level that does not suppress androgen secretion, although the mechanism of this effect is unknown. Even in cases where there is no demonstrable effect of androgen deprivation on survival, DES can decrease bone pain in as many as two-thirds of late-stage patients experiencing pain from bone metastases.

About 70% of patients with prostate cancer develop bone metastases, most frequently to the red bone marrow of the spine.²⁰⁹ Metastatic disease is a major clinical challenge since it is associated with a poorer long-term prognosis. The mechanism by which prostate cancer cells metastasize to bone is not well understood, but recent results suggest that metastatic prostate cells adhere specifically to endothelial cells lining the blood vessels within bone marrow. When rat prostate cancer cells were injected into the general circulation of a group of rats, 100% of these rats developed metastases in the bones of the spine. When cultured prostate cancer cells were exposed to a variety of cells, most cancer cells adhered preferentially to endothelial cells derived from bone marrow. Cancer cell adhesion to marrow endothelial cells was 3.5-fold higher than adhesion to other types of bone cells, and 7.5-fold higher than to liver endothelial cells. This suggests that metastatic prostate cancer cells adhere to proteins expressed on the surface of marrow endothelial cells, and that these “adherin” proteins are not expressed on the surface of liver endothelial cells.

Recent evidence suggests that the function of the tumor suppressor gene p53 is lost in prostate cancer cells.²¹⁰ The p53 gene was mutated to a nonfunctional form in three of five cultured prostate cancer cell lines and one of two primary prostate cancers. When a normal p53 gene was transfected back into those cell lines that had mutant forms of p53, growth of the transfected tumor cells was suppressed. Cells with the normal or “wild-type” p53 gene were prevented from growing in an unregulated manner, suggesting that the p53 gene has an important role in suppressing development of prostate tumors. In fact, this gene now appears to be the single gene that is most frequently mutated in human cancer; p53 gene inactivation or loss is involved in cancers of the colon, lung, breast, brain, and bladder.

Prostate carcinoma is associated with a tumor marker, called prostate-specific antigen (PSA), that is becoming more widely used in cancer diagnosis. This marker is quite specific for the prostate, and it can be detected in a blood

sample using a very sensitive assay system. PSA is secreted by epithelial cells of the prostate, whether those cells are benign or malignant. Elevation of serum PSA has been reported for carcinoma of the prostate, benign prostatic hyperplasia, and prostate inflammation. Although elevated serum PSA is not specific for carcinoma, it can provide useful corroboration of a digital rectal exam. Furthermore, serum PSA levels may also be very useful in monitoring a patient for metastatic disease after surgery to remove the prostate. Since prostatectomy should remove all sources of PSA, residual PSA in the blood serum is strongly suggestive of metastatic disease.

Recently, there has been a trend toward using the PSA test as a screening tool for prostate cancer, as a part of the routine physical. One study found that PSA was substantially elevated (i.e., $\geq 10.0 \mu\text{g PSA per liter blood}$) in 1.8% of men from a large sample population of 1653 men. Of those men with substantially elevated values of PSA, 67% had prostate carcinomas that were still too small to cause symptoms. Furthermore, it was found that PSA was more sensitive to these asymptomatic cancers than were either digital rectal examination or ultrasound. Roughly 32% of the cancer cases would not have been detected if only the traditional rectal examination had been done, while 43% of these cases would have been missed by ultrasound alone. Therefore, a combination of PSA measurement and rectal examination is expected to provide a better method of detecting prostate cancer than rectal examination alone. Furthermore, many men who would be unwilling to get a digital rectal examination may be willing to get a PSA test.²¹¹

Another study evaluated changes in blood levels of PSA over time, to determine whether such changes occurred, and whether these changes could be used as an early indicator of prostate cancer. A small population of men was examined, men whose blood had been sampled and frozen for up to 25 years as a part of another research project. A total of 54 men were studied, of whom 20 had gone on to develop benign prostatic hyperplasia, 18 had gone on to develop prostate cancer, and 16 had no prostate disease. It was found that men without prostate disease showed no significant changes in blood PSA levels over time. However, men with benign prostatic hyperplasia or prostate cancer showed an increase in blood levels of PSA over time. The rate of change of PSA in the blood actually permitted subjects with benign prostatic hyperplasia to be separated from subjects with prostate cancer. In fact, blood levels of PSA changed as much as 5 years before a diagnosis of prostate cancer was made. Thus, the rate of change of PSA levels in the blood appears to be a sensitive and specific early marker for development of prostate cancer.²¹² It is probably only a

question of time before the PSA test is incorporated into the routine physical examination of all men over 40 years old.

Taxol, a drug new to the cancer clinic, is known to be effective against some ovarian cancers and malignant melanomas. This drug is presently in extremely short supply because it is obtained from the bark of the Pacific yew tree, a tree that until recently was not thought to be worth harvesting. However, recent results suggest that taxol may also be effective in treating prostate cancer.²¹³ Human prostate cancer cells can be induced to grow in immunodeficient mice, because the immunodeficiency prevents mice from mounting a normal immune response to the human tumor. When untreated prostate cancer cells are injected into immunodeficient mice, the cells form numerous metastases to the spinal column. However, when prostate cancer cells are treated with taxol before injection, the treated cells cannot establish metastatic tumors. This suggests that taxol treatment may be able to block metastasis of prostate tumor cells. Furthermore, preliminary evidence from cancer cells in culture suggests that taxol treatment may render cells unable to invade tissue as aggressively as untreated prostate cancer cells.

CHAPTER 23

Melanoma and Other Skin Cancers

OCCURRENCE

Skin cancer is by far the most common cancer among Caucasians, perhaps surpassing all other cancers combined.²¹⁴ But people of color rarely develop skin cancer, and if skin cancer does occur it is likely to affect unpigmented portions of the body (e.g., palm of the hand, sole of the foot). The exact incidence of skin cancer is unknown, since many small skin cancers are treated by a physician during an office visit and are never reported. Other skin cancers are indolent (slow-growing) and may never receive treatment of any kind. It has been estimated that roughly 50% of whites over age 65 will develop skin cancer, and that 25% of these people (or 12% of whites over age 65) will develop more than one skin cancer.

The American Cancer Society estimated that in 1990, about 777,000 new cases of skin cancer were diagnosed in the United States. Skin cancer is nearly twice as common in males as in females, and the incidence of skin cancer rises steadily with increasing age. Yet skin cancer is so common, and so often not a life-threatening disease, that cancers other than melanoma are usually excluded from the summary statistics of cancer incidence.

Basal cell carcinoma is the most prevalent form of skin cancer, accounting for about 650,000 cases per year, or 84% of all skin cancers. Basal cell carcinomas are slow-growing, locally invasive tumors that arise from basal cells, the innermost layer of cells at the base of the epidermis. Basal cells are skin stem cells, which normally cleave off new cells to regenerate all of the cell layers of the skin. This type of skin cancer is completely curable if diagnosed early in disease progression, and very few patients with basal cell carcinoma ever die of their disease.

Squamous cell carcinoma is the second most prevalent form of skin cancer, accounting for another 100,000 cases, or about 13% of all skin cancers.

These cancers arise from the squamous (scaly) cells external to the basal cell layer. This skin cancer is also usually indolent, and curable if diagnosed early in the course of the disease. However, these cancers do have a significant chance of metastasizing, and most of the 2000 deaths per year from nonmelanoma skin cancer are caused by squamous cell carcinoma.

Melanoma is by far the most serious of the various types of skin cancer, although it represents only about 3% of all new skin cancer cases. The American Cancer Society estimated that there were over 27,000 new cases of melanoma in 1990, which represents a total of 3% of those cancers that are usually tabulated (i.e., excluding basal cell and squamous cell carcinoma). Evidence shows clearly that melanoma is curable if it is caught early: in Queensland, Australia, which has the world's highest melanoma incidence, a public education program on early signs of disease resulted in cure rates over 80%. Much of the following discussion will be devoted to melanoma, since other types of skin cancer are seldom fatal and seldom receive much attention from scientists.

An upward trend in skin cancer mortality began in the 1950s, largely as the result of an increasing death toll from melanoma. In the period between 1973 and 1987, the incidence of melanoma increased 83.3%, while the mortality rate from melanoma increased 29.8%.²¹⁵ This is a stunning rise in disease incidence in a very short period of time, and analysis of this trend has been informative about the risk factors for melanoma. The trend in melanoma incidence is most pronounced in middle-aged persons, and it is quite likely that the incidence of melanoma will continue to rise sharply.

RISK FACTORS

By far the most important risk factor for the development of skin cancer is long-term exposure to sunlight, especially when exposure leads to extensive sunburn or sunburn early in childhood.²¹⁴ People with long-term chronic exposure to sunlight (e.g., sailors, farmers) are at greatest risk of contracting skin cancer, and cancer risk rises steadily with age. Skin cancer typically occurs on skin surfaces that are constantly exposed to sunlight, such as the head, neck, and hands. The greatest risk of skin cancer is seen in persons with fair skin, light blue or gray eyes, and light red or blond hair, since people with this coloring burn easily and tend not to tan. Skin cancer risk is extremely high in albinos, who are unable to produce the skin pigment melanin, and so cannot tan at all.

The link between sun exposure and occurrence of malignant melanoma is

not as clear-cut as the link between sunlight and other skin cancers. The superficial spreading type of melanoma (70% of all melanoma cases) can develop anywhere on the body surface, with a propensity to the upper back and lower legs, so this cancer is probably not caused solely by sun exposure.²¹⁶ Yet the risk of melanoma is much higher in persons with fair coloring, or in persons of Celtic extraction, suggesting that sun burning is also causative of melanoma, as it is of the other skin cancers. Risk of melanoma is increased by having had blistering sunburns in childhood or adolescence. The incidence of melanoma among whites in Arizona is nearly three times as high as melanoma incidence in a similar population in Connecticut, which again suggests that sun exposure is the culprit. Incidence of melanoma has been increasing progressively in Connecticut in recent years, from a low of 1.2 cases per 100,000 people per year in 1935–1939, to a high of 9 cases per 100,000 people per year in 1979–1980.²¹⁷ This striking increase in melanoma incidence is probably a result of changing fashions in beauty and recreational habits.²¹⁵

Sunlight is composed of a wide spectrum of different wavelengths, but is apparently only ultraviolet (UV) light that actually leads to skin carcinogenesis. Of the three types of UV light in sunlight, UV-B has been most closely linked to skin cancer. It is worth noting that UV-B light is present in the tanning lights used in salons, so repeated visits to a tanning salon can potentially induce DNA damage and skin cancer. UV-B light is comprised of those wavelengths of light that cause delayed erythema, or sunburn. UV-A light is less damaging, and UV-C light, while very dangerous, is generally absorbed by the atmosphere of the Earth. However, scientists are now concerned that loss of ozone in the upper layers of the atmosphere will eventually lead to an increased intensity of UV-C light penetrating to ground level.

Only a small percentage of skin cancers develop from causes other than sun exposure. The most notable secondary risk factors are ionizing radiation and exposure to certain chemicals. Radiation carcinogenesis in the skin can occur because of chronic exposure to X rays or gamma rays. Chemical carcinogenesis usually involves exposure to coal tar products or inorganic arsenic. Several decades ago, inorganic arsenic salts were widely used in treatment of a range of disorders, including arthritis, asthma, and psoriasis, and they were also used as agricultural herbicides. Arsenic contamination of drinking water is thus common in many parts of the world. Arsenic exposure usually leads to the development of arsenical keratoses, which are hard, wartlike, premalignant lesions on the palms and soles of the feet. These lesions can appear a decade or more after arsenic exposure, and histologically are very similar to the keratoses produced by chronic sun exposure. It is likely that

chemical and UV carcinogenesis in skin share similar mechanisms, since UV radiation can interact with chemicals as either an initiator or a promoter of skin cancer.

The skin is often the site of tumor development if the immune system is depressed. One of the most characteristic features of AIDS is its frequent association with a normally rare skin cancer called Kaposi's sarcoma, ordinarily found only in older men of Mediterranean extraction. Generally, AIDS-associated Kaposi's sarcoma is more aggressive than normal, and is seen only in AIDS patients who are already immunocompromised. Patients with depressed immune function secondary to lymphoma, or patients receiving immunosuppressive therapy to prevent organ transplant rejection, are also at an increased risk of developing skin cancer.

There are a number of hereditary diseases that increase the risk of skin cancer. For example, individuals with xeroderma pigmentosum (XP) are characterized by extreme sensitivity to sunlight, and an estimated 1000-fold increase in risk of various skin cancers. Individuals with XP have cells with an impaired ability to repair the type of DNA damage that is induced by exposure to UV light. The DNA repair ability of patients with XP may be impaired by 50 to 90%, compared with normal individuals, and the extent of impairment correlates well with skin cancer severity. Patients with XP have a very high mutation rate in cells of the skin, and usually develop basal cell carcinoma, squamous cell carcinoma, or melanoma within the first few years of life.

Dysplastic nevus syndrome is a familial condition that increases melanoma risk about 100-fold. A nevus is a benign, large, flat, irregular, pigmented lesion of the skin that usually arises early in life, and which is a precursor to melanoma in up to 40% of cases. A dysplastic nevus often resembles a miniature version of an early stage superficial spreading melanoma. Patients suffering from dysplastic nevus syndrome can have hundreds of nevi on their body, any one of which is capable of undergoing malignant transformation to become a melanoma. These patients must keep a constant vigil to ensure that a lesion that does undergo malignant transformation is surgically excised immediately. Patients with a large congenital nevus are at a higher risk of developing melanoma, even if there is only one nevus and no family history of melanoma.

Nevoid basal-cell carcinoma syndrome is an unusual hereditary syndrome that causes a great many seemingly unrelated symptoms, and whose underlying cellular defect is unknown. Skin manifestations of this syndrome include the development of multiple basal-cell carcinomas scattered over the body surface,

together with pits on the palms and soles, and multiple epidermoid cysts. An epidermoid cyst is a spherical, firm sac within the dermal layer of skin, filled with keratin and a hardened secretion from the sebaceous (sweat) gland, called sebum. An epidermoid cyst is benign, but it probably forms from a metaplastic alteration of the epithelium that normally lines sebaceous glands. In addition to these cutaneous manifestations, patients with nevoid basal-cell carcinoma syndrome are prone to develop cysts of the jaw, abnormalities of the ribs and vertebral column, calcification of the membranes covering the brain, ovarian fibromas, and malignancies including jaw fibrosarcomas and brain medulloblastoma.

Bloom's syndrome is a very rare genetic disorder that causes sun sensitivity, short stature, and immunodeficiency. Bloom's patients have an increased risk of various malignancies, including acute leukemia and carcinomas of the skin, breast, and gastrointestinal tract.²¹⁸ Cells from patients with Bloom's syndrome have an increased frequency of chromosomal breakage, together with a sharply reduced ability to repair damaged DNA.

Another rare genetic disorder called epidermolysis bullosa (EB) can also lead to an increased risk of invasive skin cancer. EB is a recessively inherited disorder that causes extensive blistering of the skin after even the slightest mechanical stress. In patients with EB, epidermal cells are so fragile that minor abrasion causes the cells to shear apart, leaving a gap in the epidermis that is quickly filled with fluid to form blisters. Skin fragility is temperature sensitive, with blistering being much worse in the hot summer months, and this blistering can be so severe that it is debilitating. Apparently, the symptoms of EB are caused by a mutation affecting keratin, a protein that forms fibers that anchor epidermis to the underlying dermis. Cancer risk may be elevated because skin cells must constantly regenerate to replace those cells damaged by mechanical shear.

NATURAL HISTORY OF THE DISEASE

Skin cancers are always asymptomatic, except for the appearance of the tumor nodule itself, until they are far advanced. Nevertheless, skin cancer is generally rather easy to recognize, since tumors are usually manifested in parts of the body that are constantly exposed to sunlight. The three major types of skin cancer produce lesions that are quite distinct in appearance.

Basal cell carcinoma typically forms a raised, nodular tumor with irregu-

lar margins, most often occurring on the upper part of the face. The usual lesion is a noninflamed, smooth, waxy nodule that appears translucent, and may have variable amounts of melanin pigment in the form of small dots. Often there is a central flattened, depressed, or ulcerated portion of the lesion, and the lesion can take the form of a crater with rolled edges. Usually some part of the nodule will appear pearly or watery, and there may be fine, dilated blood vessels visible beneath the surface of the nodule. Occasionally, basal cell carcinoma can form a flattened, irregular, reddish patch with a roughened surface, especially when the lesion is located on the trunk. Basal cell carcinoma is seldom metastatic, but these tumors can be locally invasive, causing erosion of neighboring bone and cartilage. These tumors are remarkably painless, so they are often ignored for a long period of time, permitting them to infiltrate underlying structures.

The typical squamous cell carcinoma is a painless, firm, red nodule or plaque, with or without scales visible on the tumor surface. These tumors can also take the form of a crater, but the edges of the crater do not appear to be rolled, and there is not a pearly appearance to the tumor surface. Ulceration and crusting can occur, and large tumors often ooze blood continuously or bleed easily. Often these lesions will form a hardened or horny substance called keratin, similar to the material that forms calluses. Squamous cell carcinomas frequently arise from preexisting skin lesions called solar keratoses, which are scaly, rough, red patches that occur in chronically sun-damaged skin. Although very few keratoses ever progress to carcinoma, most squamous cell carcinomas on exposed skin do arise from solar keratoses. Squamous cell carcinomas arising from keratoses have the lowest probability of metastasis (less than 2%). Squamous cell carcinomas arising from mucus membranes, burn scars, chronic ulcers, sinus tracts, or from apparently normal skin, have a much greater tendency to metastasize.²¹⁹

Squamous cell carcinoma that is still *in situ* (i.e., not invasive) is often called Bowen's disease. These lesions are typically single or multiple thickened plaques, with sharply defined edges, a brownish red color, and a varying amount of scale at the lesion surface. These tumors can resemble patches of eczema or psoriasis, but they do not respond to therapies that are normally successful against these conditions. Only about 5% of these lesions become invasive, and less than 2% metastasize, but surgical removal is advisable.²¹⁹ Bowen's disease patients have an increased risk of developing cancers of the respiratory, genitourinary, and gastrointestinal systems, especially if the Bowen's plaque develops in skin that is not exposed to sunlight. The average latent period between the onset of Bowen's disease and the development of

visceral cancer is more than 8 years. Exposure to arsenic is often the cause of Bowen's disease, and arsenic is the only well-known carcinogen that causes both skin and visceral malignancies.

Melanomas arise from a pigment-producing cell called a melanocyte, which is originally derived from nervous tissue, and whose role is to produce and distribute pigment granules to adjacent cells. Because tumors arise from cells that ordinarily produce pigment, the tumor itself is almost always darkly pigmented, with a color ranging from tan to brown to black, and sometimes including red, white, and blue colors (the flag sign). There are four different clinical forms of melanoma, each with a rather distinct pathology²¹⁶:

1. Superficial spreading melanoma—tumor spreads horizontally, with edges that are irregular and often poorly defined, and with scattered scaly areas on the tumor surface. There can also be a central pale area within the tumor margins. These tumors occur anywhere on the body, and usually afflict patients between 40 and 50 years old (60–70% of all melanomas).
2. Nodular melanoma—tumor forms a distinct brown-black nodule, with minimal horizontal spread. Tumor growth is primarily vertical, so these tumors tend to invade deeper layers of the skin early in their course, and they tend to metastasize early. There are often areas of hemorrhage or necrotic ulceration visible on the tumor surface. These tumors also occur anywhere on the body, and usually afflict patients between 40 and 50 years old (15% of all melanomas).
3. Acral lentiginous melanoma—tumor spreads horizontally, but margins are poorly defined, while the tumor is pale, irregularly pigmented, and scaly in appearance. These tumors occur on the palms and soles, and in nail beds and mucus membranes, and are diagnosed at an average patient age of 64 (10% of all melanomas).
4. Lentigo maligna melanoma—tumors arise on a preexisting melanotic freckle, usually after a long premalignant interval. These tumors spread irregularly in a horizontal direction from a raised focus of nodularity, forming a large, irregularly pigmented lesion. These tumors tend to occur on the face and are diagnosed at an average patient age of 70 (5% of all melanomas).

Benign moles are quite different in appearance from malignant melanomas, although they can give rise to, and are often confused with, tumors. Benign moles are generally small, round or oval, symmetric, evenly pig-

mented, and only slightly raised. Moles tend to enlarge gradually during puberty or pregnancy, often thickening to form a bump on the skin surface. With time, moles may develop hairs and their color may fade. Ultimately, as many as 50 years after they first appear, moles seem to fade into the skin, or grow a stalk and drop off. Gradual uniform changes in a mole are normal, particularly when those changes follow the usual pattern. But any sudden change in a mole should be immediately evaluated by a physician. This is particularly true for people with a personal or family history of melanoma, people with 100 or more moles, and those with dysplastic moles. The following warning signs in a mole are associated with melanoma and should be examined by a physician: asymmetry of shape; irregular, ragged, notched, or poorly defined margins; irregular color variations from tan to brown to black; large size (more than a quarter-inch in diameter); a scaly, rough, or pebbly surface; itching, tenderness, or irritation; or the flag sign (red, white, and blue color). Thus, in summary, melanoma warning signs include any irregularity of mole color, thickness, or contour.

If a melanoma is removed before it has invaded other tissues, it is essentially cured. But melanoma metastasizes early, often, and widely, so it is essential that suspicious moles be removed quickly. Mole removal is a simple procedure, done in a physician's office under local anesthesia, and the procedure generally leaves only a slight scar. Long-term prognosis for the patient with melanoma is closely correlated with the thickness of the tumor, with thicker tumors indicating deeper invasion, and an increased likelihood of metastasis. Melanoma commonly metastasizes to regional lymph nodes via the lymph drainage, and the bloodstream can carry melanoma cells to sites of distant metastasis, often including brain, liver, lung, and bone.

BIOLOGY SPECIFIC TO THE TUMOR

A great deal of information has been obtained recently about skin cancers in general, and melanomas in particular. This is because of a combination of several different factors: all of the early work on carcinogenesis in animals dealt with skin cancer; the accessibility of skin tumors allows them to be sampled easily for histological examination, and their indolence may even permit repeated sampling over time; metastatic melanomas are often easily detected, even when quite small, because of their intense pigmentation; and the increas-

ing incidence of melanoma has resulted in a recent increase in funding for research on this cancer.

The mechanism by which UV light induces skin cancer is a matter of great importance, because an understanding of this mechanism might enable researchers to block skin carcinogenesis. The UV-B wavelengths of light induce the most damage in the DNA molecule, and it has been known for some time that sunlight can actually cause chemical changes in DNA. UV-B light is energetic enough to break chemical bonds, or to cause abnormal new bonds to form, within the DNA molecule, and the synthesis of DNA may be temporarily inhibited immediately after sunlight exposure. Intense UV-B exposure causes a short-term depression of basal cell mitotic rate (within 1 hour), which can persist for up to 1 day. This inhibition of basal cell mitosis is usually followed by an accelerated basal cell mitotic rate within 2–3 days. The increased rate of mitosis can last for 7 to 10 days, and can result in a mild hyperplasia of the skin that may persist for 30 to 60 days.²²⁰ Despite these cellular changes, it is probable that DNA damage is fundamental to the development of skin cancer. The strongest evidence for this comes from the fact that individuals with an impaired ability to repair damaged cellular DNA (e.g., patients with XP) are at a greatly increased risk of developing all types of skin cancer.

Tumors of the epithelium generally begin as focal growths, derived from a mutated clone of cells near the base of the skin. These mutated cells grow upward and outward within the confines of the epithelium, and it is often several years before tumor cells invade adjacent structures. As mutated epithelial cells proliferate, they compete for the limited nutrients available, and only the fittest of the cells survive. Thus, there is a potential for clonal evolution during the course of progression of intraepithelial neoplasia. Since clonal evolution can select for cells that are invasive or metastatic, eventually such cells will be produced,²²¹ and the tumor will become more malignant.

Melanoma cells can become increasingly refractory to growth inhibitors, and increasingly sensitive to growth stimulators, as a function of tumor progression.²²² When melanoma cells are mixed in culture with irradiated normal skin cells, the proliferation rate of the melanoma cells is usually suppressed if the melanoma cells were obtained from early stage nonmetastatic lesions. But if the melanoma cells were derived from late-stage lesions, then the growth rate of these cells is stimulated by growth with normal skin cells. Thus, skin cells probably release a growth inhibitor that is only effective on early stage melanoma cells, while they release a stimulatory factor that is only

effective on late-stage melanoma cells. Sustained availability of both inhibitory and stimulatory growth factors would provide metastatic cells with a growth advantage over their benign counterparts, thereby facilitating clonal dominance of metastatic cells.

Molecular biologists have studied expression of the p53 tumor suppressor gene in biopsy samples taken from skin cancer patients. Mutations of the p53 gene were detected in 50% (7 of 14) of basal cell carcinomas examined.²²³ A wide range of melanomas from different stages of tumor progression were also examined for expression of mutant p53.²²⁴ Overall, 85% (45 of 53) of melanoma specimens had detectable mutations of p53. But there was a greatly increased prevalence of p53 mutation in metastatic melanoma, compared with benign lesions or small primary melanomas. In benign dysplastic nevi, none of the samples showed mutations of p53; in small primary tumors (<1.5 mm), 60% of the samples showed p53 mutations; in large primaries (>1.5 mm), 73% showed p53 mutations; and in metastatic tumors, 93% showed p53 mutations. Thus, mutation of p53 is probably associated with progression of melanoma from an early stage to a metastatic tumor. There is even recent evidence to suggest that UV-B light may specifically affect p53 expression,²²³ so that UV-exposed cells proliferate more rapidly.

Recent evidence suggests that UV radiation of the skin is directly immunosuppressive by several different mechanisms.²¹⁹ Certain proteins on the surface of immune cells are destroyed by UV radiation, making these cells unable to respond to an antigen. The ability of immune cells to respond to an antigen can also be impaired because UV-damaged cells cannot properly process antigen. Exposure to UV light can stimulate formation of suppressor T cells, which are able to prevent the rejection of UV-induced skin tumors in mice. Finally, UV light can deplete the skin of certain cells that are involved in allergic responses.

The immune system probably plays a key role in progression of melanoma. If a primary tumor is surgically excised, there is usually a long disease-free interval during which secondary melanomas do not occur. Yet, if a second tumor does arise, it typically grows rapidly, suggesting that tumor growth rate was initially suppressed, but that growth suppression then failed. Tumor growth suppression would likely be mediated by an immune response, and failure of that immune response would permit progression of the secondary tumor. There is also evidence to suggest that partial spontaneous regression of certain types of melanoma is rather common. Superficial spreading melanomas frequently have a nonpigmented center, and microscopic evaluation of these pale areas usually indicates an absence of melanoma cells and the occasional

presence of immune cells, suggesting that the tumor has undergone spontaneous regression. Patients in whom such an immune response can be demonstrated generally have a better long-term prognosis, implying that the immune system is also able to keep secondary tumors in check. In addition, it is possible to identify melanoma patients with widely metastatic disease in whom a primary tumor cannot be found. The most likely explanation for this is that the primary tumor spontaneously regressed after seeding distant metastases. Finally, melanoma is one of the few tumors in which complete spontaneous tumor regression has been documented,²¹⁶ suggesting that there is a critical ongoing interaction between melanoma cells and the immune system of the patient.

It may become possible in the future to manipulate the human immune system, either to reveal the presence of occult metastases or to augment the immune response to established tumors. A recent demonstration of these principles used radioactively tagged antibodies to image melanoma metastases.²²⁵ Scientists created a radioactive antibody fragment that reacted with an antigen commonly expressed on the surface of melanoma cells. This antibody was administered to 20 patients, and a gamma camera was used to image sites of antibody binding, which were presumably metastatic melanomas. Antibody binding successfully detected 136 of 172 previously identified metastases, meaning that the technique was sensitive to 79% of the metastatic lesions identified. The radioactively tagged antibody detected an additional 47 sites of possible metastasis, of which 28% proved to be previously undetected tumor. While this is encouraging, it still means that 72% (34 of 47) of the possible sites of metastasis were in fact false diagnoses, being merely sites of scar tissue or chronic inflammation. In addition, nonspecific uptake of the antibody occurred in the kidney, gallbladder, bowel, thyroid, and heart, and 69% of patients developed an immune response to the radioactive antibody itself. Clearly, there is a long way to go before this technique will become clinically useful, yet there is hope for the future.

Advanced melanomas are typically extremely resistant to both radiotherapy and chemotherapy. Recent research has revealed several properties of the tumor that may, in part, account for the resistance of malignant melanoma to therapy. The pressure exerted by interstitial fluids within human melanomas is dramatically higher than the pressure of fluid in normal tissue. Interstitial fluid pressure (IFP) was measured in melanomas and in normal skin with a needle electrode that was inserted into the tissue.²²⁶ The IFP of normal skin is typically near zero, indicating that there is no significant pressure opposing the movement of blood through skin, and no significant accumulation of fluid from

blood. However, the average IFP in melanoma was 14.3 mm Hg, and measured values ranged between a low of 2 and a high of 41 mm Hg. The mean IFP was significantly higher in large tumors (22.8 mm Hg) than in small tumors (5.8 mm Hg), showing that IFP increases as the tumor grows. Mean IFP in these tumors was so high that blood flow through the tumor should be greatly reduced.²²⁷ This would result in extensive tumor hypoxia, with a consequent attenuation of tumor radiation response, and reduced delivery of cytotoxic drugs to the tumor. But if IFP within the tumor could be artificially reduced, this could result in a significant enhancement of melanoma response to radiation or chemotherapy.

CHAPTER 24

A Conceptual Overview of Cancer

It is extremely difficult to give a conceptual overview of cancer without making dangerous generalizations and oversimplifications. Any overview should consider both solid tumors and leukemias, and must be inclusive of tumors with a very broad range of individual characteristics. Necessarily, such an overview will be somewhat idiosyncratic and partially biased in viewpoint, which may offend some, enrage others, and truly please no one. Yet it is necessary to think in broad terms, since scientists must determine whether there are unifying principles of oncology and whether these principles can give insight into new cancer therapies in the future. It is also imperative to maintain the intellectual roots of oncology in cell biology, so that oncology will not become increasingly overspecialized and fragmented.

WHAT IS CANCER?

Cancer can be defined as any neoplasm that is both invasive and metastatic. Unless a neoplasm has both properties at some point in its natural history, it is benign and should not be considered a cancer. Yet this definition begs the question, since it defines cancer by its properties, rather than by its underlying causes. Such a definition is useful, but ultimately inadequate: the Fourth of July is not about fireworks, even though fireworks are the major manifestation of this holiday in the United States.

There is clear-cut evidence that virtually every cancer arises from a single transformed cell that divides innumerable times to establish a large clone of cells. This clone of cells forms a neoplasm that, to meet the definition of cancer, must be both invasive and metastatic. Therefore, instead of asking “What is cancer?” it may be more appropriate to ask the question in terms of the single

cell that produced the neoplasm. In other words, what properties of a single cell enable it to form an invasive and metastatic tumor?

A great deal of recent work shows that cells capable of forming a tumor generally have one or more activated oncogenes. Transfection of a premalignant cell (i.e., a noninvasive, nonmetastatic immortal cell) with an activated oncogene is necessary and sufficient to cause that cell to become malignant. Altered oncogene function has deleterious effects on an otherwise-normal cell, no matter how oncogene function is disrupted. This suggests that activation of a cellular oncogene (or inactivation of a tumor suppressor gene) can enable a cell to form a tumor. If the function of each oncogene could be determined, then we would know what cellular properties are causative of cancer, and not merely correlative with the cancerous state.

Cellular oncogenes are a diverse collection of genes that are critical for the regulation of normal cell growth and differentiation. Most cellular oncogenes code for proteins that are either growth factors or growth factor receptors, while some cellular oncogenes code for proteins that directly control progress through the cell cycle.²²⁸ There is a great deal of evidence showing that cancer is often associated with a genetic mutation that affects the expression of a growth factor or growth factor receptor. For example, abnormal expression of platelet-derived growth factor (and its receptors) has been documented in a large fraction of sarcomas and brain tumors. Similarly, large amounts of fibroblast growth factor (FGF) are expressed by melanoma cell lines, but not by normal melanocytes; normal melanocytes are dependent on exogenous FGF in order to proliferate, while melanoma cells need no exogenous FGF. The *ras* oncogene, which is aberrantly expressed in 30–50% of lung and colon carcinomas, and perhaps as many as 95% of pancreatic carcinomas, apparently codes for a membrane receptor involved in cell growth stimulation. These examples suggest that cancer can be induced by overexpression of one or more proteins that stimulate growth of a tumor founder cell. If these oncogene products are causative of cancer, and if they are proteins involved in cellular growth regulation, then the conclusion is simple: cancer is a disease caused by abnormal cellular growth regulation.

Defining cancer as a disease of altered growth regulation is likely to be controversial, because many scientists have argued that cancer is actually caused by impaired cellular differentiation. This assumes that cellular maturation is somehow blocked, and that cancer arises from the failure of cells to terminally differentiate. To a certain extent, this is a chicken-or-egg question: all cancers have, to a greater or lesser extent, both altered cellular growth

regulation and altered cellular differentiation. Anaplastic tumors are comprised of cells that are usually dividing rather rapidly and are morphologically undifferentiated. Proving which of these properties has primacy may be impossible, but the fact remains that the product of most oncogenes is a protein involved in the transduction of a growth regulatory signal into the cell. The cell responds to that regulatory signal by a complex set of changes, which can involve loss of cellular differentiation, an increased rate of cellular division, or both.

The absence of differentiation seen in most tumor cells could be a consequence, not a cause, of cancer. To clarify this point, let us consider a simple thought experiment: If the cells of a malignant neoplasm could be induced to differentiate fully, without the growth rate of those cells being regulated, the neoplasm would still be a cancer. But if a neoplasm could be induced to stop growing, even if the cells failed to differentiate, then that neoplasm would become benign. It may be impossible to ever actually perform this experiment, since cellular differentiation and cellular growth arrest go hand in hand. Fully mature, differentiated cells often cannot divide at all, and stem cells, which are capable of a limitless number of cell divisions, are always relatively undifferentiated. But the question of whether cellular differentiation or growth arrest has primacy in blocking tumor formation is extremely important, and a resolution of this question might provide insight into new therapeutic strategies for cancer.

A recent set of experiments seems to confirm that the absence of differentiation in tumor cells is actually a consequence of the cancerous state. Cellular proliferation and cellular differentiation appear to be mutually exclusive pathways for living cells.²²⁹ Because specific genes control each of these pathways, the decision by a cell to either proliferate or differentiate may be determined by the relative amount of gene products promoting each pathway. Thus, inappropriate expression of genes that promote proliferation might actually suppress differentiation. To investigate the possibility that a single gene controls both pathways in a reciprocal manner, researchers used a cultured tumor cell line, called 3T3-L1. This cell line has the potential to differentiate into fat-storage cells, but instead it overexpresses the *myc* gene, an oncogene involved in cell cycle regulation. The *myc* gene codes for a DNA-binding protein that becomes more abundant when quiescent cells are induced to proliferate, and less abundant when actively growing cells enter quiescence. Another gene, called enhancer binding protein (C/EBP α), also codes for a DNA-binding protein, but this protein is expressed when fat cells differentiate. Premature

expression of C/EBP α in proliferating fat cells causes these cells to differentiate and cell growth is suppressed. Yet C/EBP α is not a general growth suppressor because it is not found in quiescent fat cells. To determine whether expression of C/EBP α is sufficient to induce cell differentiation, an active form of the gene that encodes C/EBP α was transfected into 3T3-L1 cells. Transfected cells were able to differentiate into mature fat cells despite the continued presence of the *myc* gene product. Normally, expression of C/EBP α is blocked by *myc*, but when 3T3-L1 cells are forced to express C/EBP α protein, the blockage of cellular differentiation is overcome. These results show that both *myc* and C/EBP α are important in regulating fat cell growth and differentiation. However, the *myc* gene suppresses expression of the C/EBP α gene, and thereby blocks cell maturation.²²⁹ Thus, altered cellular growth regulation, not blocked cellular differentiation, is the primary lesion in these tumor cells.

To say that cancer is a disease of altered cellular growth regulation does not imply that all cancers grow rapidly. Acute myelogenous leukemia (AML) cells have an average doubling time of 7 days, whereas the average doubling time of normal hematopoietic cells is 18 hours.²³⁰ Thus, normal hematopoietic cells grow more than nine times as fast as AML cells, so AML is not a disease caused by too-rapid cell proliferation, but rather is a disease of unregulated cellular proliferation. Uncontrolled cellular proliferation probably results because leukemia cells are unresponsive to the factors that modulate growth and maturation of normal hematopoietic cells. After hematopoietic stem cells divide, the daughter cells normally go through a complex process of further cellular division with progressive differentiation, as the cells develop into mature blood cells. Leukemia occurs when stem cell progeny fail to mature further, so that they are never able to assume their normal function, yet they are able to continue dividing as would a stem cell. Such malignant cells typically produce a large number of abnormal daughter cells, so leukemia is characterized by an overabundance of one or a few primitive cell types in the blood. Yet these primitive cell types are apparently able to mature under the appropriate conditions, as we shall see.

LESSONS FROM LEUKEMIA

The idea that cancer results from aberrant or blocked differentiation of cells has been an important theme in cancer research for many years. The prevailing view has been that malignant transformation usurps the normal

program of cellular development, leading to anarchy at the cell and tissue level. However, recent evidence from the study of leukemia suggests that blocked cellular differentiation is not a universal feature of leukemia. Furthermore, the appearance of blocked differentiation may actually result from a selection for cell types that are ordinarily transitory or rare.²³¹ But the factors that result in selection and amplification of clones of leukemia cells appear to remain subservient to the normal processes of cellular differentiation.

Leukemia has been a particularly fruitful field of study because the blood of an individual patient can be sampled repeatedly, to monitor disease progression or the response of the disease to treatment. The availability of monoclonal antibodies that bind specifically to different subsets of blood cells has enabled scientists to precisely identify the different types of cells in the blood and to measure their abundance. Censusing of blood cell types has been automated, and the combination of monoclonal antibodies and automated cell sorting has revealed much about leukemia. Automated cell sorting has been used to compare the cell types present in various forms of leukemia with the cell types present in normal blood and blood-forming tissues. Normal hematopoiesis (blood formation) is now reasonably well understood, and the pathologies that can affect the process are under active investigation.

When normal hematopoietic tissues are compared with blood samples from patients with acute lymphoblastic leukemia (ALL), a similar composite pattern is often seen. For example, blood samples from patients with T-cell ALL can resemble samples of cells from the normal pediatric thymus.²³¹ The only difference is that cells that comprise no more than 2% of the pediatric thymus form the dominant cell type in blood from patients with T-cell ALL. No cases have yet been seen in which a leukemia cell expressed proteins that were unique to that tumor cell: every leukemia antigen thus far identified is also routinely seen in some normal cell population as well. The dominance of immature cell types in ALL simply reflects the fact that malignant transformation is affecting a stem cell population that is undifferentiated. Therefore, the abnormal collection of cell types seen in leukemia is the result of clonal selection for cells that are ordinarily present, but numerically rare, in hematopoietic tissue.²³¹

Underlying the apparent randomness of cell types present in leukemia, there is a surprising degree of order. Lineages of cells show fidelity in their development, even though their ultimate development may be incomplete.²³¹ The normal pattern of gene expression in lymphoid precursor cells seems to be conserved in leukemia cells. Hematopoietic renewal of the blood after massive

blood loss can mimic leukemia by temporarily increasing the number of transitory cell types. Lymphoid precursor cells in regenerating marrow can be indistinguishable from ALL cells, and a poorly regulated immune response can resemble lymphoma. The fact that leukemia involves the stabilization of cell types which are normally transitory implies that the primary lesion is regulatory in nature. Yet the fact that leukemia cells do not deviate from the normal developmental pattern (even though they may not complete development) suggests that these leukemia cells remain subservient to the normal processes of cellular differentiation.

A recent study showed that leukemic mouse cells cultured in the presence of a “differentiation factor” were capable of differentiating into mature macrophages.²³² This suggested that a leukemic state in a mouse may be caused by the inability of the mouse to generate an appropriate differentiation factor. It was found that differentiation factor could suppress the growth rate of leukemia cells in culture by as much as 93%. This differentiation factor was apparently important in the living animal as well: organ fluids extracted from 21-day-old rats were able to induce leukemia cell differentiation, while organ fluids from 7-day-old rats were not. Organ fluids from older rats thus had a higher concentration of differentiation factor than organ fluids from young rats. If 7-day-old rats are injected with cultured leukemia cells, the rats quickly succumb to disease, while 21-day-old rats are able to reject the same tumor cells. Finally, if leukemia cells are treated with differentiation factor from an older rat before injection into 7-day-old rats, the young rats are able to reject the tumor cells. This clearly shows that differentiation of leukemia cells requires an appropriate level of differentiation factor, and that, in the absence of this factor, transplanted leukemia cells retain the ability to divide and grow indefinitely.

CELLULAR DIFFERENTIATION AND CANCER

The idea that tumor cells irreversibly lose their ability to differentiate is apparently false. Many tumors are comprised of cells that are partially differentiated. For example, adenocarcinoma of the colon typically produces glandlike structures that resemble normal colonic glands, and tumor cells can even form mucus as normal colon cells do. Several other tumor types are comprised of cells that can assume a highly differentiated appearance. For example, teratocarcinoma is a malignant tumor that occurs most frequently in the testicles, where it arises from a cell that produces sperm. Testicular teratocarcinoma is

highly unusual in that histological examination of the tumor mass often reveals tumor cells that have differentiated into a wide range of different cell types. Parts of the tumor can form structures that resemble muscles, nerves, cartilage, intestinal wall, hair, nails, or even teeth. Another type of tumor, called choriocarcinoma, is a highly malignant, and frequently lethal, tumor of the testicles. This tumor can differentiate into tissues that resemble the normal tissues of the human placenta, and choriocarcinoma cells often secrete a potent hormone that is usually secreted by the placenta in pregnant women. Although both teratocarcinoma and choriocarcinoma are highly unusual, the fact that these tumors can differentiate into a range of complex structures shows that cancer is not a disease of blocked cellular differentiation.

Further evidence that cellular differentiation is not irreversibly lost by tumor cells comes from a study of cultured neuroblastoma cells. Neuroblastoma, a relatively common pediatric cancer of the adrenal gland, arises from nerve cell precursors. Neuroblastoma cells can be grown in culture quite easily, but the cultured cells bear no resemblance to neurons. Yet, if certain growth factors are added to the culture medium, the tumor cells differentiate to a form that closely resembles neurons.²³³ In the differentiated state, tumor cells are connected to each other by long strands of cytoplasm that resemble the cell processes of mature neurons. Differentiated neuroblastoma cells manufacture a protein that is typically found only in normal nerve cells. These changes in differentiation of neuroblastoma cells are reversible, and neuroblastoma cells can be cycled back and forth between a differentiated form and an undifferentiated form merely by changing the concentration of growth factor in the medium. Formation of neuronlike cell processes is not dependent on changes in gene regulation, since formation of these processes does not require synthesis of any new RNA. Apparently, genes for the differentiated state are always turned on in neuroblastoma cells, but the action of these genes is somehow blocked. Neuroblastoma is notable as the tumor most frequently involved in spontaneous regression. The responsiveness of cultured neuroblastoma cells to exogenous growth factors suggests that growth factor manipulation could be used in patient therapy, to induce regression of an established tumor.

Furthermore, certain highly anaplastic cancer cells can be induced to undergo differentiation to form normal-appearing cells; studies with cultured leukemia cells show that treatment with low doses of cytosine arabinoside can induce tumor cells to differentiate. Another growth factor, called granulocyte-colony stimulating factor (G-CSF), given to myeloid leukemia cells in culture, can induce these cells to fully differentiate and cease dividing. Treatment with

G-CSF can extend the life span of leukemic mice from less than 2 months, the life expectancy without treatment, to more than 6 months. If G-CSF is administered to leukemic mice for only 28 days, the mouse life span is extended threefold relative to a control group, suggesting that leukemia can be cured if replicating cells are induced to differentiate and cease growth.

Therefore, in summary, a cancer is a mass of cells that fails to respond properly to growth regulatory signals and that disseminates from the tumor site by invasion and metastasis. If additional features can be found that are common to all cancers, this may help us to identify properties vulnerable to therapeutic interference.

WHAT FEATURES ARE COMMON TO ALL CANCERS?

Cancer is a disease that predominantly afflicts older individuals, and more than half of all cancers are diagnosed when the patient is older than 65. This suggests either that cancer induction takes a very long time, or that older individuals are somehow inherently more vulnerable to the mutational changes characteristic of cancer. There is, in fact, some evidence to suggest that the rate of mutation of cells from older individuals is higher than that of cells from younger persons. However, there is a great deal of evidence suggesting that cancer usually takes a long time to progress from a transformed cell to a metastatic carcinoma. Epidemiological data suggest that the interval between carcinogen exposure and development of systemic cancer can be 20 to 50 years.

Tumor formation is typically a lengthy process that occurs in several phases, including an induction phase, a carcinoma *in situ* phase, an invasive phase, and a dissemination phase.²³⁴ The induction phase is when carcinogen exposure occurs, and a large amount of epidemiological evidence suggests that cancer induction alone can take 15 to 30 years. During the induction phase, carcinogen exposure results in a progressively more severe cellular dysplasia, which eventually forms a carcinoma *in situ*. There are only two notable exceptions to the generalization that cancer induction takes many years: radiation-induced leukemia can be induced in less than 2 years, and the hereditary cancers of infancy (e.g., retinoblastoma) may be present at birth.²³⁴ The likelihood of inducing cancer is probably determined by factors such as: the type, amount, and concentration of carcinogen; the duration of exposure; the presence of other carcinogens or cocarcinogens; the site(s) at which the carcinogen acts; and the individual (genetic) susceptibility to cancer induction.

The long period of time involved in the induction phase of cancer further suggests that carcinogenesis always involves multiple mutational events.

The carcinoma *in situ* phase is that period of time during which a cancer is present, but before the cancer becomes invasive. Usually this means that an epithelial cancer has not yet penetrated the basement membrane underlying the transformed cells, but it can also mean that a tumor is surrounded by a capsule that separates tumor cells from normal cells. Our knowledge of the duration of the *in situ* phase comes primarily from the study of cervical cancer, for which a widely used screening test is available. A Pap smear can detect cervical carcinoma *in situ*, in which there is an increased rate of cell proliferation and some cellular atypia, well before there is invasion of tumor cells into the cervix. The patient age at onset of carcinoma *in situ* is about 10 years less than the age at onset of invasive carcinoma, which implies that the *in situ* phase of cervical cancer lasts about 10 years. Although there are less data about other cancers, it has been estimated that the *in situ* phase of tumor progression can last between 5 and 10 years.

The invasive phase of tumor progression is that period of time during which a carcinoma *in situ* penetrates the basement membrane and begins to invade locally. Access is gained to lymphatic channels and to blood vessels, paving the way for the final dissemination phase, yet the disease is still local during the invasive phase. Tumor cells generally move along the path of least resistance during invasion, and tissues such as bone, cartilage, arteries, and nerves may provide a barrier to invasion. Eventually, tumor cells spread to regional lymph nodes, or undergo metastasis to distant sites, but the invasive phase may last from a few months to 5 years before dissemination occurs.

The final phase of cancer is dissemination, which can last for a few months up to 5 years, depending on the aggressiveness of the cancer. This phase is one of the most poorly understood, because metastases are initially microscopic in size and virtually impossible to detect. About 50% of all cancer patients probably have occult metastases at the time of diagnosis and treatment. Tumor dissemination is ultimately the cause of most cancer fatalities, since the majority of primary tumors are operable at the time of diagnosis.

The major lesson to be learned from this description of the phases of cancer progression is that there is ample opportunity to detect cancer if sensitive screening tests can be developed. The *in situ* phase provides an interval during which a screening test might detect cancer well before the tumor has progressed to be life-threatening or metastatic. As mass screening tests become more sensitive, and early detection of cancer becomes more cost-

effective, there will be a significant improvement in the cancer cure rate. The potential of this strategy for success is shown most clearly by the reduction in mortality from cervical cancer that has followed introduction of the Pap test. It is quite likely that widespread use of the Pap test could virtually eliminate cervical cancer as a cause of death. But this is not an isolated example, as experts have estimated that the mortality from breast cancer could be reduced by up to 30% if mammography were used as a routine screening test.²³⁴ Even something as simple as public education can improve the early diagnosis of cancer and significantly reduce cancer mortality: in Queensland, Australia, a public education program on early signs of melanoma has resulted in cure rates of over 80%.

A tumor mass cannot exceed a size of about 1 mm in diameter unless it is able to induce the growth of new blood vessels into the tumor mass. In fact, the major difference between a carcinoma *in situ* and an invasive carcinoma may be that the latter tumor has succeeded in inducing new blood vessel growth (angiogenesis). Since a tumor cannot form a significant mass unless it is successful at angiogenesis, it would seem that blocking angiogenesis might be a viable therapeutic strategy. If physicians could systemically block tumor angiogenesis in a newly diagnosed cancer patient, this might prevent the growth of occult metastases. Although angiogenesis has been the subject of a great deal of basic research, and blocking angiogenesis remains an attractive clinical goal, there is little clinical success to report for this approach so far.²³⁵

In order for a tumor to invade into surrounding tissue, it must be able to degrade extracellular matrix. Extracellular matrix comprises the supportive mesh surrounding each cell of the body, and also makes up the basement membrane underlying the epithelium. Matrix breakdown has been studied in cultured tumor cells for many years, and compounds have been identified that can successfully reduce the invasive ability of tumor cells in various lab assays. Prevention of matrix degradation by tumor cells in the cancer patient has nevertheless remained an elusive clinical goal.

Generally, tumors that reach the dissemination phase are comprised of cells that have an increased degree of cellular motility. Motility of tumor cells is enhanced by various motility factors, which can stimulate dispersion of tumor cells away from the primary tumor site, and toward areas where nutrient availability is better. The direction and even the rapidity of tumor cell migration can be determined by chemoattractants released by normal organs. Chemoattractants may also play a role in determining the organs to which a particular tumor cell can metastasize. If some means could be found to block activity of

those molecules that serve as tumor cell chemoattractants, then tumor metastasis might be prevented. This idea, while attractive, has not yet been tried as an approach to the clinical problem of tumor metastasis.

Another property that may be shared by most human tumors is that at least a portion of the tumor is depleted of oxygen. This occurs because the flow of blood through the tumor is often relatively slow, or because the structure of the vasculature induced during tumor angiogenesis is relatively aberrant. The net result is that some tumor cells are hypoxic, which is important for several reasons. Hypoxic cells lack sufficient oxygen to perform aerobic respiration, and anaerobic respiration is metabolically much less efficient than aerobic respiration. Thus, cancer cells are often very wasteful of the metabolic substrates they extract from blood. In addition, tumor hypoxia can have important implications for the success of both radiotherapy and chemotherapy. Radiation therapy kills tumor cells more effectively when cells are well oxygenated, because much of the cell-killing effect of radiation results from production of singlet oxygen. If tumor cells are hypoxic, singlet oxygen cannot be produced and the number of tumor cells killed by radiation is attenuated roughly threefold compared with the number of cells killed in an aerobic tissue. Finally, in order for chemotherapeutic drugs to kill tumor cells, those drugs have to reach the tumor cells, usually via the bloodstream. If a tumor is poorly perfused, there may be cells within the tumor mass that are never exposed to a chemotherapeutic agent, even if very high drug levels are attained in the bloodstream.

It is likely that an increased awareness of the extent of hypoxia in human tumors will have an impact on tumor treatment. Radiation is less likely to be used as primary therapy for large tumors if these tumors are hypoxic. Chemotherapy might be used in combination with an attempt to artificially increase tumor perfusion. Recently there has been increased interest in using chemotherapeutic agents that are preferentially toxic to hypoxic cells.²³⁶ In addition, there have been experiments in animals using chemicals that diffuse into hypoxic tumors and that act to sensitize hypoxic cells to radiation.²³⁷

HOW DOES LOSS OF CELLULAR GROWTH CONTROL PRODUCE AN INVASIVE, METASTATIC TUMOR?

As we have seen, the evidence is fairly strong that cancer is a loss of cellular growth control: oncogene mutation is often necessary and sufficient to

cause cancer; oncogene products are usually growth factors or growth factor receptors; oncogene transfection causes cellular transformation in cultured cells; and oncogene-transfected cells are uninhibited by normal cellular growth controls. However, cells that are proliferating rapidly because of oncogene action still lack the two key traits of malignant cancer, namely, invasiveness and metastatic ability.

It is thus important to ask: how does the loss of cellular growth control lead to production of tumor cells that are both invasive and metastatic? The short answer to this question is that evolution of tumor cells can occur in any tumor, and that evolution favors the production of highly aggressive tumor cells. However, this short answer deserves a more complete explanation, because clonal evolution is critically important in the biology of cancer.

After a tumor has been induced, tumor cells undergo a somewhat stereotyped pattern of change. Since a tumor almost always originates from a single tumor cell, tumor cells are initially identical in their genetic makeup. Yet evidence shows that cells that proliferate rapidly are inherently more prone to mutate. This leads to the production of many genetically mutated cells, with a wide range of metabolic and biochemical traits. Relatively few of the newly produced tumor cells can survive the rigorous conditions they encounter within a tumor, such as the relative lack of oxygen and nutrients. But if a particular trait increases the ability of a cell to survive within the tumor mass, then cells with that trait will be more likely to produce progeny. If the trait that favored survival of a mother cell can be passed down to the daughter cells, then those daughter cells are also more likely to survive and leave progeny (Fig. 13). Eventually, this process forms a new clone of cells that can potentially achieve numerical dominance over other cells in the tumor, simply because they are better at surviving and reproducing. The genetic makeup of a tumor thus changes progressively over time, which amounts to a process of tumor evolution. This process can occur very rapidly because the survival rate of newly produced cells within a tumor is often very low.

The process of clonal evolution favors the production of hardy, weedlike cells that proliferate rapidly. It has been estimated that between 50 and 80% of all newly produced tumor cells die, and in tumors such as malignant lymphoma, the cell death rate may be as high as 94%. There is a merciless competition among tumor cells to select out cells able to tolerate the harsh conditions within a tumor mass. Some of the surviving tumor cells are likely to become more aggressive than the original founder cell was, with a greater ability to induce the growth of new blood vessels and a greater tendency to

invade and metastasize. It is quite likely that clonal evolution is responsible for generating all of the features characteristic of an aggressive cancer.

WHAT INDUCES CLONAL EVOLUTION IN TUMORS?

Since very few of the cells within a tumor mass are able to survive and reproduce, this suggests that tumor cells experience very harsh selective pressures. It is important to determine what conditions within the tumor act as selective pressures, and what results in the death of most of the newly produced tumor cells. We have already noted that the supply of both oxygen and nutrients (e.g., glucose) within a tumor is reduced, because of poor tumor perfusion. Several different facts suggest that oxygen is more likely to become unavailable to tumor cells than is glucose. The normal per capita consumption rate of oxygen by cells is substantially higher than the per capita consumption rate of glucose. Furthermore, oxygen is unable to diffuse through tumor tissue as easily or as far as glucose. Finally, the oxygen-carrying capacity of tumor blood is more likely to be reduced (by acidic tumor pH) than is the glucose-carrying capacity of tumor blood.

If oxygen is scarcer than glucose within tumors, then tumor hypoxia may be the selective force that causes clonal evolution of tumor cells. This conclusion must remain tentative for now, because there has been relatively little experimental work on the subject. But a number of different characteristics of a tumor are associated with, and can be evoked by, severe hypoxia. Some of these characteristics can even be produced in nontransformed cells exposed to severe hypoxia:

1. Scarcity of oxygen induces the expression of an angiogenic factor. Under hypoxic conditions, normal macrophages can synthesize a protein that causes endothelial cells to proliferate and form new blood vessels.²³⁸
2. Transient exposure of cultured cells to severe hypoxia can evoke DNA overreplication.²³⁹ However, this point is very controversial because subsequent experiments in living mice showed that cells in an intact tumor probably do not undergo large-scale DNA overreplication. Therefore, the hypoxic conditions that exist in tumors may not induce cells to undergo DNA overreplication to the same extent as is achieved in cultured cells subjected to hypoxia.²⁴⁰

3. Transient hypoxia enhances the mutation rate of certain noncancerous cells.²⁴¹ Normal rat fibroblasts cultured under anoxic conditions induce an enzyme (endonuclease) that digests chromosomal DNA, which could account for the propensity of anoxic cells to undergo gene mutation.²⁴² However, these findings are also controversial, and other researchers have found no DNA overreplication or mutation in mouse tumor cells during and after hypoxia.²⁴³
4. Hypoxia depresses the activity of at least six different mitochondrial enzymes involved in aerobic respiration in normal cells,²⁴⁴ which should produce cells with a tumorlike anaerobic respiration.
5. Hypoxia can induce ectopic hormone secretion by tumor cells. For example, secretion of erythropoietin is induced by hypoxia in cultured renal carcinoma cells.²⁴⁵
6. Hypoxia increases radioresistance in certain cultured normal cells. The radiation response and cell-cycle distribution of these cells were characterized after cells were cultured under conditions of long-term hypoxia. There was an accumulation of cells in resistant phases of the cell cycle, cells eventually stopped cycling entirely, and cultured hypoxic cells showed an extreme radioresistance after 72 hours.²⁴⁶ This radioresistance was largely based on cell cycle distribution, rather than simply the inability of radiation to form activated oxygen species.
7. Hypoxia is often associated with lactic acid production in tumors.²⁴⁷ Lactic acid is implicated in causing a number of different effects within a tumor: inhibition of cell proliferation, DNA synthesis, and glycolysis; increased radioresistance; increased resistance to chemotherapy; and enhanced cell killing by hyperthermia.
8. Hypoxia enhances the metastatic potential of mouse tumor cells. Cultured mouse tumor cells exposed to severe hypoxia afterward show a transiently but dramatically increased metastatic potential.²³⁹

Thus, many different features characteristic of tumors may, in part, be evoked by hypoxia. Since hypoxia appears to be a theme common to virtually all malignancies, selection of drugs that are selectively toxic to hypoxic cells is a promising general therapeutic strategy for cancer.

CHAPTER 25

Cancer Therapy

Prospects for the Future

In this chapter we will attempt to survey recent advances in cancer therapy that have had a significant impact on cancer cure and patient survival. We then briefly survey possible cancer therapies in the future. This discussion is necessarily speculative; all of the therapies discussed seem to have real potential, but no one can know to what extent this potential will be fulfilled.

RECENT ADVANCES IN CANCER THERAPY

Within the very recent past a new surgical technique has been introduced, called laparoscopic surgery or “video surgery,” which will have a major impact on many different types of surgery. It is very likely that video surgery will find many new applications in the surgical treatment of cancer, and that video surgery will largely replace conventional surgery in certain situations. Video surgery relies on several recent advances in medical engineering, including improved video technology and the continuing miniaturization of electronic equipment.

Using video surgery, the surgeon can avoid making the large incisions through skin, muscle, and ligament that are characteristic of most thoracic and abdominal surgeries. Instead of making one very large incision into the body, which causes a great deal of postoperative pain and a long convalescence, a series of small incisions are made near the region of interest. These small incisions enable the surgeon to view the internal organs on a video screen, to insert various surgical instruments into the thoracic or abdominal cavity, and to perform the surgery. For example, video surgery to remove the gallbladder requires four small incisions in the abdomen, each only about an inch long. One

incision near the navel is used as an entry point for a gas line, which is used to pump carbon dioxide into the abdomen. This causes the abdominal muscles to lift away from the internal organs, so that the surgeon can gain easier access to the organs. A second small incision serves as an entry point for a video camera, which is about the size and shape of a cigarette lighter. The video camera is attached to a video monitor by fiber-optic light cables, so that images from within the abdomen can be magnified (up to 18-fold), and projected onto a television screen during the operation. In addition, fiber-optic light guides serve as a conduit for light into the abdomen, to illuminate the operative field. Through the remaining two small incisions in the abdomen, the surgeon can insert various surgical instruments for cutting, stapling, and sewing tissue, while the movement of these instruments is viewed on the video monitor. Surgery under these conditions is much like playing a video game, since the only visual feedback to the surgeon comes from the video screen. Video surgery has become firmly established as the surgery of choice to remove the gallbladder: in 1991, a total of about 600,000 video gallbladder operations were performed in the United States, or about 80% of all gallbladder surgeries. Video surgery of the gallbladder reduces the typical convalescent period of the patient from 6 or 8 weeks down to as little as 1 week. With video surgery, patient time under anesthesia is reduced, there are fewer postoperative complications (e.g., infection, postoperative pain), the surgical incisions are reduced from one scar 8 inches long to four scars of about an inch each, and the typical hospital bill is reduced by more than \$1000. It is likely that video surgery will enjoy a substantial increase in use in surgical oncology, for various problems including lymph node biopsy or removal, lobectomy of the lung, partial or total hysterectomy, removal of the pancreas or prostate, partial or total colon resection, and liver biopsy.

There has also been striking improvement in the effectiveness of chemotherapy for several of the major cancers. The mortality for Hodgkin's disease declined by 50% between 1973 and 1987, even though disease incidence declined only 16%.²⁴⁸ Nearly 50% of patients with Stage IV Hodgkin's disease can now be cured, whereas this stage was uniformly fatal two decades ago. The decrease in mortality from this cancer is largely a result of the introduction of combination chemotherapy. Combination chemotherapy uses several different chemotherapeutic agents administered simultaneously, with the drugs carefully chosen so that each functions by a different cell-killing mechanism. This increases tumor cell kill while reducing the likelihood of selecting for drug-resistant clones of tumor cells. The introduction of new chemotherapeutic agents has also increased the cure rate for some cancers. For example, use of

cis-platinum has resulted in an improved prognosis for patients with testicular seminoma: the cure rate for Stage III patients before *cis*-platinum was 20–60% (depending on the volume and location of metastases), but complete, sustained remission can now be obtained in 70–80% of Stage III cases. Several new chemotherapeutic regimens, now in clinical trials, show promise in treating cancer of the colon, rectum, uterus, ovary, and breast.

Radiation is still widely used in the treatment of early stage cancer, or to prevent local recurrence of advanced stage disease. There have been several improvements in radiation therapy, including radiation dose fractionation (and hyperfractionation), use of high-voltage, proton, or neutral particle beam radiation, and use of radiation sensitizers for hypoxic tumors.²⁴⁹ The introduction of radiosurgery has resulted in better palliation of large intracranial lesions and even some apparent tumor cures. Radiosurgery uses tightly focused radiation to deposit high doses of radiation in a small volume of tumor while sparing surrounding brain. Computerized, three-dimensional treatment planning allows clinicians to combine medical images from different modalities (X-ray computed tomography and magnetic resonance imaging) to obtain accurate three-dimensional renderings of a tumor in an organ. This enables improved radiation treatment planning, to spare the organ while dosing the tumor with the maximum possible radiation dose. This has had an impact on the success of radiation therapy for cancers of the prostate, liver, brain, and cervix. There is now an effort to determine how well a tumor is perfused before radiation treatment, so that patients with hypoxic tumors can be spared the rigors of radiation therapy (Fig. 56). It is anticipated that continued improvements in computer systems, and in medical imaging systems, will allow further improvements in treatment planning.

However, despite the recent successes of existing cancer therapies, there is much room left for improvement. Current chemotherapy and radiation therapy are simply too good at killing all cells to be truly specific for tumor cells. The concept of therapeutic ratio is very useful in this context: this is a ratio of the total number of tumor cells killed to the total number of normal cells killed by a particular therapy. Current therapies are relatively nonspecific to tumor cells, so these therapies have a low therapeutic ratio. Ideally, treatments should have a high therapeutic ratio, and kill many more tumor cells than normal cells. Current chemotherapeutic agents have such a low therapeutic ratio that treating a tumor with chemotherapy is much like doing surgery with a steak knife: it can be done, but many normal cells are killed. Maximum therapeutic dose to the tumor is limited by normal organ toxicity, meaning that the patient is treated to the limit of his tolerance, but the tumor is probably still not completely killed.



Figure 56. Magnetic resonance imaging (MRI) optimized to show blood flow to tumor and surrounding brain (contrast with Fig. 26, from the same patient during the same exam). A pediatric patient (11 years old) was imaged to determine the extent of brain tumor recurrence 4 months after surgery for glioblastoma. This image was acquired after injection of a blood-borne contrast agent, and brightness of an area is proportional to the rate of contrast flow into that area. Bright regions correspond to volumes of brain through which blood flow is rapid, while dim regions correspond to less well-perfused volumes of brain. Three bright spots are visible in the midline of the brain, which are major arteries or veins. The area of excised primary tumor (lower right) is dark, implying slow perfusion, while the area of recurrent tumor (lower left) is brighter, implying faster perfusion than even many normal brain regions. Above the bright region (lower, left) is a volume of tumor that is poorly perfused and

therefore unlikely to respond well to radiation therapy. (Photograph courtesy of Dr. June Taylor, Dr. V. Eugene Reddick, and Dr. James Langston, St. Jude Children's Research Hospital.)

The impetus is therefore to develop therapies that are selectively toxic to cancer cells, or that at least have a better therapeutic ratio.

CANCER THERAPY IN THE FUTURE

Recent advances in genetic engineering now enable oncologists to synthesize recombinant toxins for use against tumor cells. Recombinant toxins are molecules made by recombinant DNA technology, which combine a cell-targeting protein with a potent cell toxin.²⁵⁰ The cell-targeting protein could be a growth factor known to bind selectively to specific cancer cells, or it could be an antibody against a specific tumor antigen. The toxin could be any of a

number of different molecules that are highly toxic to all cells, such as bacterial toxins (e.g., *Pseudomonas* exotoxin, diphtheria toxin) or plant toxins (e.g., ricin from castor beans). However, a strong chemical bond between the toxin and the cell-targeting protein limits the cell-killing ability of the toxin so that it is selectively toxic only to tumor cells. In principle, cells that do not bind the cell-targeting protein are completely spared the cell-killing effect of the toxin. The goal is that a recombinant toxin should target a feature of the tumor cell that is unique to that cell, so that normal cells are not killed by the toxin. Since recombinant toxins kill cells by mechanisms that are different from conventional chemotherapy, cross-resistance to chemotherapeutic agents should be eliminated. Furthermore, recombinant toxins are not mutagens, so they should not induce secondary malignancies and should not cause benign malignancies to progress. In principle, recombinant toxins can be mass-produced at low cost (in bacteria) and may be able to provide a high therapeutic ratio.²⁵⁰

However, the practical problems in making and using recombinant toxins are legion. Although many cancer cells overproduce growth factor receptors that promote the growth of the cancer cells, many normal cells display identical receptors.²⁵⁰ For example, epidermal growth factor receptor (EGFr) is present in large amounts (up to 3×10^6 or 3,000,000 receptors per cell) on the surface of many squamous cell carcinomas, glioblastomas, and some metastatic ovarian and bladder cancers. But normal cells display up to 3×10^5 receptors per cell, so there is only a 10-fold higher level of EGFr density on tumor cells. In fact, there are probably no antigens that are expressed exclusively by tumor cells. Most antigens on tumor cells are also expressed on several to many normal cell types throughout the body, so that a specific antibody could kill normal cells as well as tumor cells. Furthermore, in order to limit the toxicity of recombinant toxins exclusively to tumor cells, the chemical bond between the cell-targeting protein and the cell toxin must remain intact. Otherwise the toxin could be liberated from the targeting protein so that the toxin would become generally toxic. Very few chemical bonds are stable for long periods of time in the blood, so this is a formidable problem. Finally, recombinant toxins are foreign to the body and are highly immunogenic (i.e., cause an immune response). In the absence of immunosuppression, a patient could potentially generate an immune response to a toxin within 10 days. This would limit the amount of time that a particular recombinant toxin could be used in the treatment of a tumor.²⁵⁰

Despite these problems, there are several recombinant toxins that are currently in clinical trials.²⁵⁰ The currently active trials all use immunotoxins (a

combination of antibody and toxin) and all fall into one of two broad categories. The first type of clinical trial uses immunotoxins to kill tumor cells in bone marrow aspirate, before the marrow is injected back into the cancer patient. This kind of treatment (called autologous bone marrow transplantation) is only used for patients with leukemia. The second type of clinical trial uses immunotoxins infused into the patient that are intended to localize to the site of the tumor. This kind of treatment is usually reserved for patients who have failed alternate therapies, and who have a large tumor burden, but the range of cancers currently being treated in this way is fairly broad (e.g., melanoma, colorectal carcinoma, breast cancer, ovarian cancer, and lymphoma). Definite responses to immunotoxin treatment have thus far been seen only in certain lymphoma patients.²⁵⁰

Biologic therapy can be defined as any cancer treatment that acts primarily through the natural defense mechanisms of the patient, or by the administration of substances that naturally occur in the patient.²⁵¹ There have already been some notable successes with this technique: interferon- α is now the treatment of choice for hairy cell leukemia, while hematopoietic growth factor is commonly used to augment red blood cell production in cancer patients undergoing chemotherapy.²⁵² Certain bioactive molecules, such as interferon- α and interleukin-2, can be directly toxic to cancer cells. Biologic therapy has benefitted enormously from recent advances in molecular biology and biotechnology that have made it possible to obtain a wide variety of biological materials in large quantities for the first time.

Biologic therapy can be based on manipulation or modulation of the immune system, in a process called immunotherapy, or on the ability to introduce new genes into cells, in a process called gene therapy. Modulation of the immune system is now better established than gene therapy in the treatment of cancer, but both therapeutic approaches are evolving so rapidly that it is impossible to predict which will achieve more success in the future. Many tumors are only weakly immunogenic, so there has been an effort to increase the antigenicity of tumor cells. Efforts to increase tumor immunogenicity or stimulate an immune response have included unmasking antigens on the tumor cells, infecting tumor cells with viruses, and inducing the expression of viral antigens on the cell surface by interferon treatment.

Most current efforts at immunotherapy of cancer have taken an approach called adoptive immunotherapy. Adoptive immunotherapy is defined as injection into the patient of immunoactive agents, including activated cells of the immune system, which directly or indirectly stimulate an immune response.²⁵¹

Recently several cell types have been identified that can cause tumor regression in some patients, and these cells can be raised in large quantity in cell culture. The most potent immune-active cells identified thus far are lymphokine-activated killer (LAK) cells and tumor-infiltrating lymphocyte (TIL) cells, both of which are activated T cells. The difficulty of obtaining these cells in large quantity has largely been overcome with the discovery of interleukin-2, which stimulates growth of activated T cells in cell culture. Current immunotherapy is capable of inducing prolonged regressions in selected patients with advanced metastatic disease.²⁵¹ It is impossible to guess whether these isolated instances of success represent a major advance in the offing, or whether they will remain as isolated instances, but it is clear that immunotherapy is worthy of fuller exploration in the future.

Gene therapy, the introduction of new genes into cells, is a cancer therapy that is still in its infancy. Nonetheless, recently a group of oncologists at the University of Michigan received approval from the Food and Drug Administration to begin a clinical trial of a new therapy for patients with malignant melanoma. The gene chosen for gene therapy was a histocompatibility gene, which encodes a protein normally involved in cell recognition by the immune system. Hundreds of thousands of copies of this gene will be injected directly into the tumors of melanoma patients who have responded poorly to conventional therapy. Normally, injection of a gene into a tumor mass would not enable the gene to reach the interior of the tumor cells, so that the gene could not be expressed. To circumvent this problem, each gene will be encased within a liposome, a tiny sac made of lipid that can actually fuse with the cell membrane, releasing the gene into the cell cytoplasm. If the histocompatibility gene is then expressed by the tumor cell, this might induce the immune system of the patient to recognize the tumor as foreign, much as the immune system recognizes a transplanted organ as foreign. This ingenious strategy could potentially induce tumor rejection, in a manner analogous to organ rejection.

The problem of how to smuggle a gene into a tumor cell, and how to ensure that the gene is expressed once it gets there, is truly daunting. Many researchers have tried many different strategies, all with relatively little success. It seems that the best strategy would be to use viruses as a gene vector, since viruses are able to induce normal cells to take up and express viral genes. Yet viruses are so small that there is very little room for a therapeutic gene to be packaged within the viral particle. Furthermore, unless the viral genes are removed or inactivated, the patient may become infected with the viral genes as well as the therapeutic genes. This could be a major problem in a patient whose

immune system may be functioning poorly in the first place. Recently a group at the University of North Carolina tried a novel approach. They used an adenovirus, which normally attacks cells of the epithelium lining the respiratory tract, but all of the viral genes within the viral particle were inactivated or deleted. This did not affect how the virus entered the cell, since none of the viral genes are needed to induce cells to take up the viral particle. Furthermore, instead of cramming the therapeutic gene inside the viral particle, the gene was attached to the outside of the virus using a chemical linker. In tests with cultured human cells, the viral particle was taken up easily, via a receptor on the cell surface, and the therapeutic gene was expressed at high levels. Because the therapeutic gene was on the outside of the viral particle, it was nearly sevenfold larger than it could have been if it were contained within the virus. But this success has not yet been achieved with cells in a living animal, so it is unknown whether the technique will ultimately be useful in the clinic. If this innovative technique could be used to introduce and express therapeutic genes in targeted human cells, this could be a major advance. The technique may also prove to be very flexible, since virtually any gene could be attached to the outside of the viral particle using a chemical linker.

The definition of biologic therapy, as therapy that uses substances that naturally occur in the patient, may be too restrictive. There are several potential therapies that exploit natural bodily processes, but that rely on administration of drugs that do not occur naturally in the patient. For example, an increased understanding of the role of growth factors in cancer development could enable clinicians to combine biologic therapy with chemotherapy. Growth factors might be used to synchronize tumor cell division, so that a population of tumor cells would become more vulnerable to cell cycle-specific chemotherapeutic agents. Stimulation of cell division often involves a cascade of biological events, one of which is phosphorylation of tyrosine in certain proteins. A molecule called tyrphostin, which can block tyrosine phosphorylation, inhibits the growth of cultured tumor cells at a concentration that causes little cell toxicity. There have also been efforts to directly block the action of oncogene products. The *ras* oncogene codes for a protein that is localized to the cell membrane, but if this protein is prevented from moving into the membrane, cell growth is inhibited. Alternatively, the action of *ras* protein can be inhibited with certain small proteins that bind to the *ras* protein and inhibit cell growth.²⁵² It is also possible that tumor growth stimulation by growth factors could be blocked directly, using antibodies that bind specifically to a growth factor receptor, so that the receptor is blocked or down-regulated. This ap-

proach has proven successful in limited tests in cultured cells and in experimental animals.

A completely new approach to blocking tumor growth has been developed recently, which relies on blocking the expression of specific genes within tumor cells. The genetic information used to synthesize a protein is stored in the DNA molecule. This information is first transcribed into a molecule of RNA before protein can be synthesized using RNA as a molecular template. The RNA molecule thus transcribes genetic information from the DNA molecule and translates it into a protein. It is now possible to inactivate specific RNA molecules that carry the genetic code for specific proteins. This technique uses what is called antisense RNA, strands of which bind to RNA molecules with a specific "sense" sequence. The bound sense RNA molecule is thus inactivated, while all other RNA molecules are undisturbed, meaning that a specific protein can be selectively deleted from a cell (Fig. 57).

The power of the antisense RNA technique was demonstrated recently in experiments using a cultured human breast tumor cell line. Researchers used cultured human breast tumor cells (MCF-7) that are dependent on added

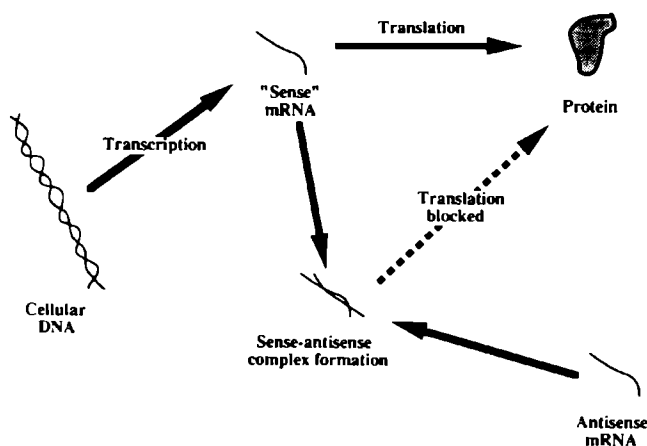


Figure 57. Diagram of the way in which antisense RNA can be used to selectively delete the product of a single gene. Normally a gene is transcribed from the cellular DNA into a molecule of messenger RNA (mRNA), which is indicated here as "sense" RNA. This mRNA is then translated into a specific protein, as shown at the top of the diagram. However, if a piece of RNA that is exactly complementary to the "sense" RNA is introduced into the cell, this antisense RNA will bind to the sense mRNA, thereby rendering it unable to serve as a template for translation of protein. This selectively blocks translation of that one mRNA only, so the effect is the same as if a specific gene was deleted from the chromosome.

estrogen for cell growth.²⁵³ When estrogen was added to MCF-7 cells, it induced expression of the *c-myc* gene. Yet the *c-myc* gene was also constantly expressed in a second breast tumor cell line that did not require estrogen added to the culture medium. This suggests that *c-myc* expression is required for growth of breast tumor cells, whether or not those cells are dependent on estrogen. When *c-myc* expression was specifically blocked in MCF-7 cells using antisense RNA, this blocked the ability of these cells to respond to estrogen. Normally, hormone-deprived MCF-7 cells respond to estrogen by inducing a fivefold increase in *c-myc* protein expression within 90 minutes. When *c-myc* antisense RNA was added to MCF-7 cells before the cells were exposed to estrogen, the synthesis of *c-myc* was inhibited by 95%, and cell growth was inhibited by 75% over 9 days. The antisense RNA for *c-myc* even inhibited the growth of breast tumor cells that were estrogen-independent. This confirms that *c-myc* expression is required for the growth of both estrogen-dependent and -independent breast tumor cells, and shows that inhibition of *c-myc* expression can result in long-term suppression of tumor cell growth. Since breast tumors often progress to estrogen independence, loss of estrogen regulation of the *c-myc* gene may be an important factor in cancer progression. These findings are very important because *c-myc* amplification is seen in 22–32% of human breast tumors, where it is generally correlated with poor prognosis.²⁵³ It may eventually become possible to specifically inhibit tumor growth in a breast tumor with antisense RNA directed against the *c-myc* gene.

CANCER SCREENING

There have been clear indications that screening programs can lead to early detection of cancer, and it has long been known that early detection is the best way to obtain a cancer cure. Therefore, widely available, broad-spectrum screening tests will likely become an important element of cancer therapy in the future. There is now good evidence of the potential for screening programs to reduce cancer mortality. For example, screening programs for liver cancer have been in place since the early 1970s in the Far East, where there is a very high incidence of hepatocellular carcinoma. Large numbers of individuals have been screened, to identify asymptomatic patients, because tumor resectability, operative mortality, and prognosis are far better for asymptomatic liver cancer than for symptomatic cancer. Current screening tests enable clinicians to identify liver cancer patients about 14 months before they would become

symptomatic. The screening programs in the Far East have substantially reduced mortality from liver cancer among screened individuals, suggesting that such programs should be emulated in the United States, at least among certain high-risk populations. Furthermore, there was a 40% reduction in mortality from cervical cancer in the years between 1973 and 1987, with most of this reduction attributed to early cancer diagnosis by the Pap test. A Pap smear can detect carcinoma *in situ* as much as 10 years before there is any invasion of tumor into the cervix.

The benefits of screening for colon cancer were highlighted by a recent study that examined the diagnostic utility of several different colon cancer screening tests. Colon cancer arises from fingerlike polyps on the inner wall of the intestine that can take 5 to 10 years to form tumors, so screening tests are designed to locate polyps before they become cancerous. A new, flexible, fiber-optic viewing instrument, called a sigmoidoscope, can be used to view the last 2 feet of the colon, where roughly half of all colon cancers arise. This instrument is able to identify more polyps than the old-fashioned rigid sigmoidoscope, which cannot be inserted as far into the colon. Routine use of a flexible sigmoidoscope reduces colon cancer mortality by 60% for cancers arising in that portion of the colon that can be visualized, which translates into a 30% overall reduction in colon cancer mortality (since half of all colon cancers arise beyond the point that can be viewed by even the flexible sigmoidoscope). Patients who are not examined by sigmoidoscope are significantly more likely to die of colon cancer. A group of colon cancer patients who had died of their disease was compared with a control population who did not have colon cancer, and it was found that only 8.8% of the colon cancer patients had been screened by sigmoidoscopy, while 24.2% of the control population had been screened for colon cancer.²⁵⁴ Although sigmoidoscopy can be very effective at identifying colon cancer when it is still treatable, this raises some difficult sociological issues. Experts have estimated that providing all Medicare patients with colon cancer screening by sigmoidoscopy, together with the necessary treatment in patients identified by the test, could cost up to \$2 billion a year. While this is a tremendous monetary cost, there are also monetary, social, and ethical costs in allowing the poor to die of treatable cancers.

The introduction of sensitive screening tests for cancer susceptibility could enable susceptible individuals to selectively reduce their exposure to carcinogens to which they are especially sensitive. For example, a recent study showed that individuals carrying one copy of a gene for ataxia-telangiectasia (AT) are more prone to develop cancer after radiation exposure.²⁵⁵ These

individuals show no signs or symptoms of AT, because expression of AT requires two defective AT genes to be present, but they are nevertheless more prone to suffer genetic mutation after exposure to medical X rays. About 1.4% of the population of the United States, or between 2 and 3 million people, are believed to be carriers of the AT gene, so a large number of people are at risk. Individuals with AT (i.e., who have two mutant copies of the AT gene) have a cancer risk that is elevated about 100-fold relative to a control population. But individuals who have only one copy of the defective AT gene are still at an elevated risk for all cancers; for men the relative risk is elevated 3.8-fold, while for women the risk is elevated 3.5-fold. However, female breast cancer risk specifically is elevated more than 5-fold. If women with a defective AT gene could be identified by a screening test, then these women could avoid radiation exposures such as mammograms. Since it has been estimated that 9 to 18% of all breast cancer patients carry a defective AT gene, this could result in 5000 to 10,000 fewer breast cancer cases per year.

Finally, another measure that could substantially improve the overall cure rate for various cancers is to increase the equitability of health care distribution. The American Cancer Society estimates that blacks have a 7% greater likelihood of contracting cancer than whites, but they have a 27% greater likelihood of dying from their cancer, probably because of limited access to adequate health care. The poor, the uninsured, and the underinsured have a higher risk of being diagnosed with an advanced-stage cancer, and of receiving inadequate treatment once that cancer has been diagnosed. Late diagnosis of cancer is frequently a consequence of inadequate routine health care; poor patients often do not have regular physicals and may postpone a visit to a physician until very serious symptoms are present. Even after these patients have been diagnosed, they have a significant chance of receiving inadequate health care. A recent study of women with breast cancer showed that women at small urban hospitals, which often serve a large population of poorly insured patients, are likely to receive substandard health care.²⁵⁶ The study followed 5766 patients with breast cancer in 99 Illinois hospitals, or roughly 84% of the estimated new breast cancer cases in the entire state that year. Most of these patients received a partial or complete mastectomy as primary treatment for the cancer, but nearly half of the patients did not receive appropriate adjuvant therapies. On average, 48% of patients with early stage tumors did not receive radiation therapy after a partial mastectomy, while 44% of these patients did not receive hormone therapy or chemotherapy. In 9% of patients who received a mastectomy, even a simple dissection of adjacent lymph nodes was omitted from the treatment plan.

These treatments are now regarded as appropriate for all patients with early stage breast cancer, in order to improve local disease control and prevent systemic metastasis. Adjuvant therapies were most likely to be omitted at small urban hospitals that service a large fraction of underinsured patients. Thus, poor patients are likely to receive inadequate cancer therapy at their neighborhood hospitals, and are ultimately more likely to die of their cancer. Until health care access is equal for all people, the poor will continue to suffer needlessly and will often die of curable cancers.

Advances in symptom management, pain control, and psychosocial counseling of the patient suggest that symptom palliation and long-term cancer control may become a viable alternative to cure in some cases. Most chronic diseases, such as hypertension, coronary artery disease, diabetes, arthritis, Parkinson's disease, ulcerative colitis, manic depression, and schizophrenia, are not curable. However, disease progression can be arrested and symptoms can be palliated instead. Similarly, it may be unreasonable to expect cancer to be curable in every case. If disease progress can be arrested, while symptoms are controlled, then even an inoperable cancer might become a chronic disease. The goal of cancer control in such a case would be to reduce cancer to a condition that might limit a person's activity but would not significantly shorten expected life span.

CANCER PREVENTION

When cancer death rates are adjusted for age, factoring in the greatly increased life expectancy of recent years, it becomes clear that there has been no increase in the overall incidence of cancer between the years of 1930 and 1985.²⁵⁷ The alarming increase in incidence of lung cancer has been offset by a mysterious decline in several other cancers, including cancers of the stomach, liver, and uterus. If lung cancer, which is almost entirely a disease of smokers, is removed from the calculations, then the incidence of cancer overall is stable or declining. This is more than likely the result of recent efforts at cancer prevention. Nevertheless, there is no room for complacency, because if the present rates continue, 20% of us will die from cancer.

There has been a greatly increased emphasis on cancer prevention in recent years, and several different lines of evidence indicate that 50 to 80% of all human cancers are potentially preventable.²⁵⁸ Cancer prevention, or at least a substantial reduction in cancer incidence, may become possible if several

relatively simple strategies are implemented. The first strategy is to identify specific causes of human cancer, through epidemiological studies of cancer risk factors and through laboratory studies of carcinogenic mechanisms. The second strategy is to increase our intervention in the carcinogenic process. This might include risk factor reduction, cancer vaccination, or chemoprevention of cancer. The final strategy is to increase the sensitivity and availability of screening and early detection methods for the common cancers. By combining these separate elements into a unified program of cancer prevention, it is quite likely that there could be significant reductions of cancer incidence and cancer mortality within the next decade. However, a unified program of cancer prevention will not occur without a significant increase in commitment and leadership at the national level.

Risk factor reduction is a term that denotes the reduction of exposure of individuals to known carcinogens, and the identification of unknown carcinogens, so that those exposures can also be minimized. Risk factor reduction is very difficult to achieve, because it requires modifying individual behaviors, as well as the activities of corporations and public institutions. Tobacco use alone accounts for roughly one-third of all cancers in the United States, and it has been estimated that, globally, tobacco causes 2.5 million deaths per year.²⁵⁸ There have been antismoking campaigns in the United States for more than 20 years, yet there are still about 56 million American smokers. The relative lack of success of programs directed toward smoking cessation indicates that individual behaviors are as difficult to change as corporate behaviors. Since occupational and environmental exposure to chemical carcinogens probably accounts for no more than 5% of all cancers in the United States, corporations are not the major culprit.²⁴⁸ Rather, life-style patterns are the largest single contributor to cancer incidence, with diet alone estimated to account for 20 to 60% of all cancers in the United States.²⁵⁸ However, there have been several hopeful signs of change in life-style patterns: individual consumption of dietary fat in the United States has fallen steadily since 1965; changes in food preservation practices have been mandated by law in the United States; and there is an increased awareness of the importance of regular physicals and screening tests such as the Pap test.

Cancer vaccination is an option that has been little explored until now, but there are some indications that vaccination could be effective against certain cancers.²⁵¹ There have been efforts recently to genetically modify tumor cells, in order to force these cells to induce a more rapid or effective immune response. Enhancing the expression of certain genes, such as the major

histocompatibility complex (MHC) antigens, can increase the immunogenicity of tumor cells in experimental animals. Injection of cells with enhanced expression of MHC antigens leads to production of cytolytic tumor-infiltrating lymphocytes that are not produced in response to tumor cells expressing low levels of MHC antigen. This is really a form of gene therapy, but if antigens could be identified that are widely expressed on tumor cells, this would allow cancer vaccination to become a reality.²⁵¹

Intervention in the carcinogenic process itself may be easier to accomplish than risk factor reduction. The malignant transformation of cells is usually associated with some mechanism that enhances the normal rate of cell proliferation. Chronic stimulation of cell division is involved in many types of cancer, including hormonal stimulation of mitosis in breast and prostate cancer, viral induction of mitosis in cervical cancer, and chemical stimulation of mitosis in lung cancer. Thus, chemicals that block the stimulation of cell mitosis may have potential as chemopreventive agents. For example, calcium blocks the proliferative effect of bile acids on the cells lining the colon, because the calcium complexes with bile to form insoluble bile salts.²⁵⁹ Calcium has been given to individuals with a family history of familial polyposis, in an effort to reduce the rate of malignant transformation of polyps. The chemical difluoromethylornithine (DFMO) can suppress cellular proliferation by interfering with polyamine metabolism, thereby blocking a metabolic pathway required for cell growth. DFMO has been very effective in blocking tumor formation in experimental animals, and it is now in clinical trials as a chemopreventive agent in humans. Experimental work with animals has also shown that retinoids inhibit cellular proliferation, perhaps by enhancing the normal processes of cellular differentiation. Currently a retinoid (4-hydroxyphenyl retinamide) is being evaluated in clinical trials as a way to prevent cancer of the opposite breast in patients previously diagnosed with unilateral breast cancer.²⁵⁹

The most desirable effect of a chemopreventive agent would be to promote regression of neoplasia, even after cellular dysplasia or chromosomal aneuploidy is already present.²⁵⁹ This idea may not be so farfetched, since disappearance of aneuploid cells during the spontaneous regression of epithelial cancers has already been documented in human oral, colorectal, bronchial, and cervical epithelium. In one case, an aneuploid leukoplakia in the mouth was observed to regress after treatment with β -carotene. Another example is a reported regression of adenomatous colon polyps in patients with familial polyposis, induced by treatment with sulindac, an anti-inflammatory agent. A

prospective, blinded study verified that sulindac did cause complete polyp regression in six patients and partial regression in another three patients. After sulindac was discontinued, the polyps recurred in some, but not all, cases.²⁵⁹

Many chemopreventive agents are natural products that are present in certain foods.²⁵⁸ This is consistent with evidence that diet can act to decrease as well as increase cancer incidence. For example, compounds known as aromatic isothiocyanates can inhibit carcinogen activation and can also induce the expression of an enzyme involved in carcinogen detoxification. These compounds are found in relatively high concentration in cruciferous vegetables, including cabbage, broccoli, kohlrabi, Brussels sprouts, cauliflower, turnip, mustard, and radish. Vitamin C is able to inhibit the formation of the potent carcinogens known as nitrosamines, and this vitamin is present in a wide range of fruits and vegetables. β -Carotene, also present in a wide range of fruits and vegetables, is an antioxidant that may be able to inhibit the activation of certain carcinogens. Various phenols and flavones, which are present in a wide variety of plants, are also able to inhibit carcinogen formation. Limonene, a compound present in oil of lemon, orange, caraway, and dill, may be able to interfere with the action of an activated *ras* oncogene. Another compound called nerolidol, which is present in certain flower oils, appears to be able to inhibit *ras* activation.²⁵⁸

Incremental improvements in the sensitivity and availability of cancer screening tests and clinical diagnostic tools are expected to have a major impact on the success of cancer treatment in the near future. However, in our zeal to improve cancer screening and diagnosis, we must not overlook the poorer segments of our society. Ultimately, it is a failure of the system if poverty itself remains a major risk factor for cancer.

CONCLUSION

It is appropriate to conclude this text with a plea for a more broadly based attack on cancer. Clinicians cannot hope to increase the success of their treatment of cancer without ongoing input from basic scientists concerned with cancer research. But cancer researchers must be willing to accept more input from clinicians, so that research is more directly keyed to patient needs. And both clinicians and cancer researchers must be more willing to interact extensively with classically trained biologists. The science of biology and the

art of medicine must become more tolerant of each other, since the only way the patient can benefit is with a skillful combination of the two.

Despite our knowledge of the molecular biology and chemistry of cancer, we know very little about basic tumor physiology.²⁴⁹ For example, there is little practical understanding of drug delivery to tumor cells. We have a very good understanding of classical pharmacokinetics; of how drug uptake, distribution, elimination, and biotransformation affect levels of a particular drug in the bloodstream. We also have a reasonably good understanding of how a particular drug affects an individual tumor cell, and the biochemical mechanisms involved in drug action. However, we have virtually no understanding of how drugs distribute within the tumor mass, of the kinetics of drug movement to the tumor cell membrane, or of the impact of tumor hypoxia on drug distribution within the tumor.

Similarly, the processes that affect an individual tumor cell within a tumor mass are virtually unknown: Can tumor cells recover completely from hypoxia? What actually happens to a tumor cell after it receives a lethal dose of radiation or chemotherapy? How is a killed tumor cell cleared from the tumor? How does the tumor stroma respond to changes in the tumor parenchyma?

Perhaps the greatest area of ignorance is in the complex interactions between the patient and the tumor. By what mechanism is the immune response to a tumor attenuated in the cancer patient? Can the immune response be therapeutically modulated to induce tumor rejection? What are the systemic effects of cancer and how are these effects mediated by the tumor? Is cachexia ultimately a helpful or harmful response to a tumor? And finally, how does cancer kill the patient?

Currently, most cancer researchers have a narrowly focused attack on cancer, using cancer cells in culture to answer questions that are primarily molecular or biochemical in nature. At the same time, classical biologists with a broad interest in biology seldom tackle questions related to cancer. This dichotomy between cancer research and biology must be broken down, so that biologists do not feel constrained from attacking questions related to cancer, and cancer researchers do not feel compelled to limit their research to rat, mouse, and human cells in culture. Both cancer researchers and biologists need to broaden their inquiries to include new questions, new models, and new techniques in their research.

Glossary

Acute Short-term; of short duration or sudden onset; opposite of chronic.

Adenocarcinoma A malignant tumor that forms microscopic structures resembling glands; a tumor with a glandlike character when viewed in histological section.

Adenoma A benign tumor of epithelial tissue that can form glandlike structures; typically adenomas are not invasive, but they can grow to compress normal structures.

Adenopathy Swelling or enlargement of lymph nodes. Swelling can be slight and painless, as in Hodgkin's lymphoma, or it can be fairly painful, but it is usually chronic rather than acute.

Adjuvant therapy Secondary cancer therapy used as a follow-up to enhance the effect of primary therapy. For example, a chemotherapeutic agent may be used to kill metastatic tumor cells before they can form a clinically significant tumor, even if the existence of metastatic cells is undiagnosed. Adjuvant chemotherapy is used most frequently in cases where the primary tumor is likely to have seeded occult metastases by the time diagnosis is made. Adjuvant chemotherapy could also be used after surgical debulking of a tumor that cannot be completely excised.

Adrenalectomy Surgical removal of one or both hormone-secreting adrenal glands; used as therapy for certain hormone-dependent cancers such as breast and prostate cancer, because removal of the hormone source can result in tumor regression. For example, growth of the normal prostate gland is dependent on presence of the male hormone testosterone, so it is logical to attempt to halt progression of prostate cancer by androgen deprivation. About 90% of all androgens are secreted by the testes, with the adrenal glands making up the difference. Thus, therapy for prostate cancer often involves surgical removal of the testes, combined with adrenalectomy.

- Aerobic* Living in, or requiring, oxygen. Well-oxygenated tissue is often referred to as aerobic, meaning that cells can survive because there is an adequate supply of oxygen to the tissue.
- Alopecia* Loss of hair or baldness. This is a relatively frequent side effect of chemotherapy, but it usually does not persist, even in patients receiving long-term high-dose chemotherapy.
- Amino acids* The building blocks of protein; proteins are composed of a linear array of amino acids, which then fold into a unique three-dimensional shape. The properties of a protein are determined, in part, by the particular constituent amino acids and, in part, by the three-dimensional shape that these amino acids assume.
- Amplification* Gene duplication; an increase in the number of copies of one or several genes. Gene amplification is frequently seen in cancer cells, and is often manifested as double minute chromosomes, or homogeneously stained regions in a chromosome.
- Anaerobic* The absence of oxygen; living without oxygen.
- Anaplasia* The absence of normal cellular differentiation in tissues. Anaplastic cells tend to divide at a higher rate than normal cells and the cellular appearance, especially during division, is aberrant. Anaplasia is found in most malignancies and the degree of anaplasia is often the best indicator of prognosis for a tumor. Benign tumors are less anaplastic than malignant tumors, so benign tumors usually resemble the tissues from which they were derived.
- Anaplastic* Tissue characterized by anaplasia; growing without form or structure.
- Androgen* Male hormone. A generic term for a hormone, such as testosterone or androsterone, that stimulates the normal cellular activity of the male sex organs, or enables the expression of male sex characteristics such as facial hair.
- Anemia* Thinning of the blood. Any condition in which the number of red blood cells per unit volume, or the total amount of hemoglobin per unit volume, or the proportion of red blood cells per unit volume, declines. The clinical significance of anemia is that the oxygen-carrying capacity of the blood is reduced, so that tissues are more likely to become hypoxic. Anemia is frequently manifested as pallor of the skin, shortness of breath, heart palpitations, lethargy, or fatigability.
- Aneuploid* Cells with an abnormal number of chromosomes. Aneuploidy usually involves loss of one or a few chromosomes, but extensive chromosomal loss is possible.

- Angiogenesis* Development or induction of new blood vessels. The ability of tumors to induce angiogenesis is in large part responsible for their ability to survive, grow, and metastasize.
- Anorexia* Diminished appetite; food aversion. This can result in extreme physical wasting or weight loss in the advanced cancer patient.
- Antibody* A protein, secreted by plasma cells of the immune system, that interacts with and neutralizes a specific antigen. Antibodies are defined by their ability to interact with, and bind to, antigens, which are molecules that stimulate an immune response. Antibodies serve as one of the first lines of defense when the body encounters an antigen with which it has already had experience. However, if the body is exposed to a novel antigen, it can take 8 to 14 days for plasma cells to produce new antibodies that bind to the new antigen.
- Antigen* Any molecule that induces a response by the immune system, including allergens such as ragweed pollen. An antigen is defined by its ability to bind specifically to an antibody, or to induce secretion of new antibodies by plasma cells.
- Antioxidant* Chemicals, often naturally occurring, that can interact with and neutralize harmful oxygen free radicals. Antioxidants such as β -carotene, vitamin C, and vitamin E have a protective effect on cells through their ability to trap oxygen radicals. Cruciferous vegetables (e.g., broccoli, cabbage, Brussels sprouts, and cauliflower) are all high in natural antioxidants.
- Ascites* An accumulation of fluid within the thoracic or abdominal cavity. Ascites can result from obstruction of the return flow of lymph fluid to the bloodstream.
- Aspirate* To remove fluid by suction; alternatively, the substance removed by aspiration.
- Asymptomatic* Without symptoms or obvious manifestations of disease.
- Autocrine secretion* Secretion of a substance that has a self-stimulatory effect on those cells that secreted that substance. Autocrine secretion is believed to be involved in the stimulation of tumor cell growth in small-cell lung carcinoma.
- Autoimmune* An immune response to oneself. Autoimmunity is believed to be involved in the pathology of several different diseases, including diabetes mellitus, lupus erythematosus, multiple sclerosis, and myasthenia gravis. Self-reactive antibodies are normally deleted from the immune system, but occasionally, for unknown reasons, self-reactive antibodies can be produced. This results in an immune attack upon normal tissues, and can result in the destruction of those tissues.

Basophil A blood cell that stains darkly; contains granules that bind dye.

B cell B lymphocyte; a cell of the immune system that circulates in the bloodstream; once a B cell is activated, it divides and differentiates into a plasma cell, which can secrete large quantities of antibody to the antigen that stimulated the immune response.

Biologic therapy Any cancer treatment that acts primarily through the natural defense mechanisms of the patient, or by the administration of substances that naturally occur in the patient. Biologic therapy can be based on manipulation or modulation of the immune system, in a process called immunotherapy, or on the ability to introduce new genes into cells, in a process called gene therapy.

Biopsy The process of removing a small sample of tissue from a patient for diagnostic purposes; a specimen obtained by surgical sampling of tissue.

Biotransformation Degradation or conversion of a molecule from one form to another by the biochemical action of living tissue. Biotransformation can occur when an inactive (or less active) form of a chemical carcinogen is partially degraded by normal enzymes of the body to a form that is a more potent carcinogen. Alternatively, biotransformation may be required to change an inactive form of an anticancer drug to a more active form.

Blast An immature precursor cell. This word is often used as a suffix, to indicate that a particular cell type is immature (e.g., an osteoblast is an immature bone cell, a lymphoblast is an immature lymphocyte).

Blood chemistry A series of chemical tests run on a blood sample, to determine whether that sample falls within the normal range for a variety of different characteristics. A blood chemistry work-up can analyze any of over 100 different chemical constituents of blood. Some of the constituents that are commonly measured in blood include hemoglobin, oxygen, pH, carbon dioxide, sodium, potassium, calcium, urea nitrogen, and cholesterol, and can also include tumor marker proteins such as prostate-specific antigen or carcinoembryonic antigen.

Bone marrow The soft tissue in the center of bone, particularly in the long bones of the arms and legs. Bone marrow is the tissue that is responsible for replenishing cells of the bloodstream as they die. To totally replace all of the blood cells that die in a single day, the bone marrow (and lymph glands) must produce 500 billion new cells each day.

Bone scan A diagnostic test for abnormal metabolic activity of bone cells. Usually this involves injection into the patient of a radioactive material (technetium-99m) that is deposited temporarily in metabolically active

areas of bone. Subsequently, a gamma camera is used to image areas of bone radioactivity. Positive bone scans are associated with any metabolic condition that leads to elevated metabolism of bone cells, including tumor metastases to bone, healing fractures, and arthritic or inflamed sites. Bone scans can be very useful for monitoring tumor progression or assessing response of metastatic disease to treatment.

Brain stem The area of the brain beneath the paired halves of the cerebrum and the cerebellum, and connecting these structures to the spinal cord. Almost all of the sensory and motor nerves pass through the brain stem to the spinal cord, so tumors of the brain stem are associated with palsies affecting these various nerves. The brain stem is also responsible for a multitude of “primitive” functions, such as the breathing and swallowing reflexes.

Bronchoscopy Inspection of the interior of the major airways of the lung with a bronchoscope. A bronchoscope is usually a flexible, fiber-optic instrument that enables the physician to view the surface of the airways or to collect biopsy samples from lesions suspected to be cancerous. During a bronchoscopic examination, the patient receives local anesthesia, then the bronchoscope is inserted through the nose and maneuvered into the lung. The introduction of bronchoscopy has enabled clinicians to diagnose lung cancer without subjecting the patient to a surgical biopsy.

Buccal cavity The mouth; pertaining to, or adjacent to, the cheek.

Cachexia Extreme weight loss or general wasting of the body, which can be a symptom of advanced cancer. Cachexia is a general lack of nutritional adequacy, which is probably not simply the result of insufficient dietary intake.

Cancer screening Examination of a group of asymptomatic individuals, to detect those with a high probability of having cancer. The most familiar cancer screening test is probably the Pap test, which involves histological examination of cells swabbed from the cervix, to detect cells that have undergone malignant transformation.

Carcinogen Any substance capable of causing or inducing cancer.

Carcinoma Any of a large number of different types of malignant tumor derived from epithelial cells covering the surface of a tissue. The most common carcinomas are those that affect skin, large intestine (colon), rectum, lung airways, prostate gland, breast, and cervix.

Carcinoma in situ An early stage, noninvasive tumor derived from epithelial cells.

Cell cycle The life cycle of a cell; the series of events involved when a cell undergoes cell division; generally the life cycle is divided into phases as follows: gap (G_1) phase, before the cell initiates the process of cell division; synthesis (S) phase, when the cell replicates its genetic information (DNA); a second gap (G_2) phase, during which the cell prepares to undergo cell division; and mitosis (M) phase, when the cell divides to form two daughter cells.

Cellular oncogene A gene associated with cancer. Oncogenes typically become capable of inducing malignant transformation of cells only when the oncogene is mutated, or when the oncogene is aberrantly regulated so that it functions abnormally. Oncogenes are common to widely divergent groups of organisms, so it is probable that the normal function of these oncogenes is critical to cellular growth or development.

Central nervous system (CNS) The brain and spinal cord.

Cerebellum The brain mass located at the base of the skull, beneath the large paired cerebral hemispheres and above the brain stem, at the approximate origin of the spinal cord.

Cerebrum The largest portion of the brain, containing all of the “higher” functions such as speech and vision. The cerebrum, or cerebral hemispheres, occupy a major portion of the skull volume, and include all of the brain except the cerebellum and brain stem.

Chemotherapy Treatment for cancer, involving administration of chemical substances or drugs designed to kill any rapidly dividing cells, such as tumor cells.

Cholestasis An arrest in the flow of bile, caused by obstruction of the bile ducts. Cholestasis is often caused by tumors of the liver or pancreas, and is usually associated with jaundice.

Chromosome An extremely large molecule, comprised of DNA and proteins, which is really a linear array of genes. Forty-six chromosomes are located in the nucleus of every cell in the human body (with the exception of red blood cells and gametes), and each chromosome contains the genetic information needed to synthesize hundreds of different proteins.

Chronic Long-term; of long duration; opposite of acute.

Clonal evolution Progressive change through time in the genetic information carried by a clone of cells. This requires that the fidelity of replication of genetic information be imperfect, so that some cells acquire mutations that are then passed down to their progeny. The process of clonal evolution favors hardy, weedlike cells that proliferate rapidly, and is probably

responsible for development of drug resistance, tumor invasiveness, and tumor cell metastatic ability.

Clone A colony of cells, originally derived from a single cell, and all having similar or identical genetic information. In a sense, the human body is a clone, since all of the cells comprising the body are derived from a single fertilized egg. Tumors are usually described as clonal, meaning that the entire tumor is derived from a single transformed cell.

Combination chemotherapy Any therapy for cancer involving the administration of several different anticancer drugs simultaneously. The rationale is that while tumor cells may be resistant to one of the agents used, they are unlikely to be resistant to all of them, especially when the effects of the different agents are combined.

Complement A set of blood proteins that is capable of destroying certain bacteria or some malignant cells. Complement is actually a complex of at least nine different proteins that interact with antigen-antibody complexes in an extremely intricate fashion.

Complete remission Disappearance of all signs of cancer; this does not necessarily mean that the tumor is cured, it merely indicates that the tumor is no longer detectable. A tumor is considered to be cured only when it has been in complete remission for more than 5 to 10 years (depending on the particular type of cancer).

Computed axial tomography (CAT) Outdated terminology; see computed tomography.

Computed tomography (CT) Sophisticated medical imaging technique. X-ray beams are projected through the body from a variety of angles, and these beams are absorbed by hard structures such as bone. Images can be reconstructed to show fine details of hard or bony structures, viewed as if the body had been cut in cross section. Reconstruction of image "slices" avoids the ambiguity that can be associated with viewing normal X-ray images, which are projection images (i.e., showing the three dimensions of the body collapsed down into a two-dimensional image). CT is not good for imaging soft structures, and requires the patient to be subjected to low radiation doses.

Contraindication Indication against a treatment; any symptom or circumstance that makes it inadvisable to perform a particular therapy or procedure.

Cytology The study of cells; any diagnostic test involving the microscopic examination of cells shed from, or sampled from, a tumor.

- Cytopenia* A reduction or absence of certain cellular elements in the blood.
- Cytoplasm* That portion of a cell excluding the nucleus; the cytoplasm contains numerous cell organelles, such as the mitochondria.
- Cytotoxic* Detrimental to, or destructive of, cells. Chemotherapeutic agents are cytotoxic agents, which usually have an effect on any cell progressing through the cell cycle.
- Cytotoxic T lymphocyte (CTL)* Tumor-killing cells; one type of activated T cell can develop into a CTL, which directly attacks tumor cells. CTLs can only attack cells that display tumor antigens in conjunction with self antigens, so these cells are unable to attack bacterial cells, and they may have evolved specifically to eliminate tumor cells.
- Differentiation* Process of cellular specialization, during which a cell develops from a nonspecialized “stem cell” into a fully specialized mature cell; mature, differentiated cells tend to have a unique appearance and role in the body, distinct from other differentiated cell types.
- Diploid* The state of a cell that possesses the normal number of chromosomes. Most cells are diploid throughout their life span, meaning that they have two copies of each chromosome.
- DNA* **Deoxyribonucleic acid**; the molecule that is the repository of genetic information in cells; the chemical name for the information-containing portion of a chromosome, which encodes all of the genetic information necessary to make proteins.
- Dose fractionation* A particular strategy for administering radiation to a tumor; the practice of dividing up a total radiation dose over a long period of time (days or weeks), so that many separate small doses of radiation are administered. This minimizes radiation damage to normal tissues, while still providing an effective radiation dose to the tumor, because normal cells are better able to repair radiation damage than are tumor cells.
- Double minute (DM)* Little bits of chromosomal material that are not integrated into a chromosome, which indicate that gene amplification has occurred.
- Duodenum* The first portion of the small intestine. The duodenum is the site of outlet of the pancreatic duct and the bile duct, so it can become involved in cancer originating in either site.
- Dysphagia* Difficulty in swallowing.
- Dysplasia* A cell growth disorder; variation from the norm in the size and shape of cells of a particular tissue, often accompanied by loss of normal cell orientation and by an increase in the number of cells, although cell

division appears to be normal. A relatively common form of dysplasia is cervical dysplasia, a loss of organization of cells on the surface of the uterine cervix.

Ectopic hormone Inappropriate or abnormal secretion of a chemical substance that has a biological effect on a tissue or organ distant from the site of secretion, and that may affect the general health of the patient. Because hormones are such potent molecules and can elicit a very complex web of responses in tissues throughout the body, inappropriate release of hormones is a serious, potentially lethal, condition.

Edema Accumulation of an excessive amount of watery fluid in cells, tissues, or body cavities.

Endocarditis Inflammation of the membrane lining the heart.

Endothelial cells Cells that form the lining of structures such as blood vessels and the heart; typically endothelial cells form a sheetlike layer over underlying tissue.

Eosinophil A leukocyte, or cell of the blood, which stains readily with eosin dye.

Epidemiology Study of the prevalence and spread of disease in a community.

Epistaxis Nosebleed; profuse bleeding or hemorrhage from the nose.

Epithelial cells Cells that form a layer over body or organ surfaces; epithelial cells form a skin over the body surface, as well as over the surface of organs and other structures.

Epithelium Cellular layer over an organ or structure.

Erythrocytosis An increase above the normal in the number of red cells in the blood; this condition often occurs in response to a particular stimulus; also known as polycythemia.

Exfoliate To shed or slough off, as in exfoliated cells shed from a tumor mass.

Familial Involving the family; affecting several members of the same family.

Fibroblast A type of cell that can form connective tissue or cartilage.

Fibroma A benign tumor derived from connective tissue.

Fibrosis Formation of fibrous tissue; usually results from a reparative or reactive process, as in recovery from a wound or injury.

Follicular Of, or relating to, a follicle, or spherical mass of cells usually containing a cavity.

Fragile site A portion of a chromosome that is prone to breakage or damage.

Gamma camera imaging Medical imaging, using a device that detects gamma emissions from a radioactive isotope. A gamma camera forms a two-dimensional image, usually with low resolution, of any structure

within which radioactivity concentrates; the most familiar gamma camera image is a bone scan, in which localization of technetium-99m to bone is detected.

Gamma ray A type of radiation used in radiation therapy; produced by the radioactive decay of naturally occurring elements and capable of causing ionization of chemicals or destruction of cells.

Genome The complete set of genes carried on the chromosomes of a cell.

Grade Tumor classification based on the degree of differentiation of tumor cells; can be a very important indicator of prognosis for the cancer patient; tumor grade is distinct from tumor stage, which is based on size of the primary tumor and extent of dissemination of secondary tumors.

Granulocytic leukemia Myelogenous leukemia.

Granulocytopenia Reduction in number of the white blood cells called granular leukocytes.

Granulocytosis A condition characterized by an increase above the usual number of granular leukocytes in the blood; an increased abundance of a certain type of white blood cell.

Gray A measure of radiation dose; the amount of absorbed energy per unit volume of tissue. Biologic effects of radiation are determined by the time course over which radiation is delivered and the radiation dose per fraction; the normal dose in fractionated radiation is 10 Gy per week, delivered in fractions of 1.5 to 2.5 Gy.

Growth factor Any of various molecules, usually proteins, that can stimulate or suppress cell growth rate, or regulate progress of cells through the cell cycle. Growth factors are secreted by cells or organs of the body in a very tightly regulated manner during normal growth and development; several oncogenes are very similar in sequence to growth factor genes.

Half-life The length of time required for the amount of radioactivity emitted by a radioactive material (per unit time) to decline by half.

Haploid The state of a cell that possesses half the normal number of chromosomes. Most cells are diploid ($2n$) throughout most of their life span, meaning that these cells have two copies of each chromosome. When gametes (i.e., eggs or sperm) are formed, each gamete has a haploid ($1n$) number of chromosomes, containing only one copy of each gene.

Helper T cell Cells that provide helper function to other T cells of the immune system, and that can stimulate B cells to begin their own process of maturation. Helper T cells develop from cells that are a target for the AIDS virus, so loss of helper T cells cripples the immune system.

Hematemesis Vomiting of blood.

Hematopoietic Pertaining to the formation of red blood cells; also hemopoietic.

Hematuria A condition in which the urine contains blood or red blood cells.

Hemiparesis Slight paralysis affecting only one side of the body.

Hemorrhage A flow of blood; often denoting an abrupt and massive blood flow, but can also describe chronic or internal bleeding.

Hepatoma Old terminology denoting liver cancer; now referred to as hepatocellular carcinoma.

Histology Microanatomy; study of the structure of cells, tissues, and organs by examination of prepared specimens under a microscope.

Homogeneously stained region (HSR) A region of a chromosome that lacks the normal banded appearance typical of chromosomes; this is taken to mean that gene amplification has occurred at this site on the chromosome.

Hormone Potent bioactive molecule that is normally secreted in tiny quantities by a limited number of tissues or organs in the body. Hormone synthesis and release is very tightly regulated under normal conditions, because these molecules have such a potent effect on the physiology and health of the patient. Inappropriate hormone secretion (either hormone deficiency or hormone excess) is associated with a range of very serious medical conditions, including: diabetes, dwarfism, Cushing's disease, hyperthyroidism, acromegaly, and failure to reach sexual maturity.

Hyper Prefix indicating overabundance or elevation above the normal.

Hypercalcemia An abnormally high concentration of calcium ions in the bloodstream.

Hyperchromatic Highly colored; abnormally pigmented; excessively stained.

Hyperplasia A fairly common cell growth disorder, in which there is an increase in the number of cells of a particular tissue or organ without formation of a tumor. Older men are frequently victims of benign prostatic hyperplasia, in which cells of the prostate form nodules that may partially obstruct the urinary tract. Hyperplasia of the breast can occur in younger women during pregnancy and lactation, or in older women afflicted with fibrocystic disease. In most types of hyperplasia there is an expectation that the condition will reverse spontaneously or that treatment will lead to cure, without local recurrence and without progression to malignancy.

Hypo Prefix indicating deficiency, or depression below the normal.

Hypophysectomy Destruction or surgical removal of the hypophysis, or pituitary gland.

Hypoxia A decrease of oxygen availability below normal levels.

Iatrogenic cancer Cancer induced by the therapeutic effort itself; chemotherapy or radiotherapy can induce a second primary or iatrogenic cancer, years after therapy for the first primary cancer.

Immunocompromised An individual with an impaired ability to respond to an immune challenge; the result of immunosuppression, either by agents such as chemotherapy or radiation, or by immunosuppressive effects of a tumor.

Immunogenic Stimulatory of an immune response; antigenic or allergenic.

Immunosuppression Suppression of a normal immune response; use of pharmacological, chemical, physical, or immunological means to attenuate an immune response; immunosuppression is required for most organ transplants or tissue grafts.

Immunotherapy A type of biologic therapy based on manipulation of the immune system.

Inflammation A biological response to injury; a fundamental process involved in healing, which is associated with destruction of injurious material (e.g., bacteria) and the cellular responses leading to tissue repair. Typical signs of local inflammation include redness, warmth, swelling, and pain. Redness and warmth result from an increased volume of blood at the inflamed site; swelling results from congestion of blood vessels and associated edema; pain results from physical pressure on, or stretching of, local nerve endings.

Interferon A molecule that stimulates macrophages to become involved in an immune response.

Interleukin A molecule released by macrophages that stimulates maturation of immature T cells into cytotoxic lymphocytes (CTLs); it apparently causes fever in some cancer patients.

Invasion Local spread of a tumor by infiltration or destruction of adjacent tissue.

Invasive Infiltrative, locally destructive.

Jaundice A serious medical condition, resulting from obstruction of the bile ducts, in which the skin and whites of the eyes take on a yellowish cast because of deposition of a pigment called bilirubin, which is ordinarily excreted through the bile ducts.

Labeling index (LI) The proportion of cells that take up a radioactive precursor of DNA and use that precursor to make new DNA; newly

synthesized DNA is thus “labeled” with radioactivity, so the proportion of growing cells in a population of cells can be estimated.

Laparoscopic surgery “Video surgery”; use of video technology and miniaturized surgical equipment to perform surgery remotely through small abdominal incisions. Internal organs are viewed on a video screen during surgery, and various surgical instruments are inserted into small incisions and manipulated using visual feedback from a video screen during surgery.

Leukocyte White blood cell; any one of three different classes of cells in the blood, including myeloid cells, lymphoid cells, and monocytic cells; cells involved in engulfment or digestion of foreign particles, stimulation of allergy, or generation and modulation of the immune response.

Lymph node Any one of numerous small bean-shaped bodies located along the lymph vessels; nodes are concentrated in the neck, armpit, and groin. Lymph nodes are involved in facilitating an immune response to various antigens (e.g., bacteria, viruses, metastatic tumor cells) by serving as sites of interaction between macrophages, B cells, T cells, and antigen.

Lymphocyte A type of white blood cell, formed in lymphoid tissues of the body, including the lymph nodes, spleen, thymus, tonsils, or bone marrow, and responsible for the immune response. Lymphocytes include B and T cells and represent 22–28% of all leukocytes (white blood cells).

Lymphocytic (lymphatic, lymphoid) leukemia Characterized by uncontrolled proliferation of blood-forming cells in lymph tissue located in the lymph nodes, spleen, and lungs. This results in production of large numbers of immature lymphocytes.

Lymphocytic series B cells and T cells; blood cells involved in the immune response.

Lymphopoietic Pertaining to the formation of B cells or T cells.

Lysis The destruction of cells by digestion of the cell membrane.

Macrophage Scavenger cell; any large cell found in the blood and specialized for phagocytosis, which is the engulfment and digestion of material such as dead tissue and degenerating cells.

Magnetic resonance imaging (MRI) Very sophisticated medical imaging technique. MRI uses a powerful, external magnetic field to align the nuclei of hydrogen atoms, then these nuclei are made to resonate by a radio frequency pulse. Anatomic images of exquisite detail can be reconstructed, where image contrast is provided by differences in the abun-

dance or physical state of the hydrogen nucleus; since the body is more than 80% water and the water molecule contains two hydrogen atoms, MRI is able to make beautiful images of soft structures such as brain. But MRI is not good at imaging hard structures such as bone, which do not contain much water.

Marker A characteristic by which the presence of a tumor can be recognized; often particular proteins in the bloodstream, such as prostate-specific antigen or carcinoembryonic antigen.

Melena Dark-colored, tarry stool; indicates the presence of partially digested blood in the feces.

Meninges Membranes covering the brain or spinal cord.

Metaplasia An unusual cell growth disorder; abnormal transformation of a mature differentiated tissue of one kind into a mature differentiated tissue of another kind. For example: intestinal metaplasia is the transformation of the lining of the stomach into glandular tissue resembling the lining of the intestine; Barrett's esophagitis is the transformation of the flattened cells that normally line the esophagus into columnar cells resembling cells that line the stomach.

Metastasis Migration of tumor cells away from a primary tumor, usually through the bloodstream or lymph fluid, to a distant site in the body, where a secondary tumor is initiated.

Metastatic Able to metastasize, or undergo metastasis.

Mitosis A type of cell division, which results in the production of two cells that share identical genetic information.

Mitotic figure The physical appearance of chromosomes during mitosis.

Molecule The smallest unit of a particular chemical that still retains all of the properties of that chemical; chemicals are composed of many separate molecules with identical chemical properties.

Monoclonal antibody A population of identical proteins, usually secreted by a single B cell or a clone of B cells, each of which interacts with and neutralizes the same antigen.

Monomers The molecular subunit of a polymer; polymers can be composed of several to many monomers; also, the subunits of an enzyme that can be made of several associated proteins.

Mucosa Mucous membrane; a moist membrane covering a structure.

Multidrug resistance Resistance of tumor cells to several different chemotherapeutic agents simultaneously; often seen in cases when a patient is treated with a drug that succeeds in causing a partial regression, but not a

cure, of a tumor. The regressing tumor can recur in a form resistant to a range of different chemotherapeutic agents, not just the drug that was initially successful.

Mutagen Any agent that induces mutations in cellular DNA, including chemicals or radiation.

Myelogenous (granulocytic, myelocytic, myelogenic, myeloid) leukemia Leukemia characterized by uncontrolled proliferation of blood-forming cells located primarily in bone marrow, which results in production of large numbers of immature cells in the myeloid series.

Myeloid series Blood cells involved in the immune response.

Natural killer (NK) cells A cellular component of the immune system; NK cells can attack any cell that displays a tumor antigen, whether or not a self-antigen is also displayed. This ability to kill tumor cells does not depend on prior exposure to the antigen and does not involve antibodies. NK cells are indiscriminate killers of cells recognized as nonself, and may maintain long-term surveillance of cells in the body, guarding particularly against virus-induced tumors.

Neoplasm A benign or malignant tumor; any abnormal tissue or cell mass; distinct from new tissue produced during wound healing, where new tissue precisely replicates the original architecture of damaged tissue.

Neutrophil A type of mature leukocyte, or white blood cell, formed in the bone marrow.

Nodular Forming nodules, or knotlike swellings.

Nucleic acid DNA or RNA; a family of chemicals, found principally in the cell nucleus, that are the building blocks of genes.

Nucleus The rounded mass in the center of most cells, within which chromosomes are located.

Occult Undiagnosed, hidden; usually refers to a tumor too small to be detected.

Oncogene A gene, mutation of which is associated with transformation of a normal cell to a malignant cell. Oncogenes are often similar to genes for growth factors or growth factor receptors.

Oophorectomy Ovariectomy; surgery to remove the ovaries.

Operable Condition amenable to surgery, in which surgery is expected to offer relief or cure.

Opportunistic infection Any infection or disease that occurs only when the natural resistance of the patient is lowered; since late-stage cancer typically causes some suppression of the immune system, many advanced

cancer patients suffer such opportunistic infections as thrush, candidiasis, osteomyelitis, endocarditis, and septicemia.

Osteomyelitis An inflammation of the bone marrow and adjacent bone.

Ovariectomy Surgery to remove the ovaries. Ovariectomy may be performed in patients with advanced breast cancer, since ovarian hormones stimulate the growth of many breast tumors. Alternatively, an antiestrogen such as tamoxifen may be used to inhibit the binding of estrogen to the estrogen receptor, or chemical agents can be used to directly inhibit the synthesis of estrogen.

Paraneoplastic syndrome A severe medical condition affecting the whole body, which can be associated with cancer; any systemic condition attributable to the effect of a tumor on the patient.

Parenchyma Cells specific to, or characteristic of, a gland or organ; opposite of stroma, which are cells common to many different organs, such as connective tissue or endothelial cells.

Partial remission Abatement or reduction in severity of symptoms of a disease; specifically, a reduction in volume of a tumor without tumor cure

Pathological fracture A broken bone, resulting from bone weakening by disease processes.

Pathology The study of all aspects of disease, especially the nature, causes, and course of disease processes; structural and functional changes that result from disease.

Peptide Small protein; combination of two or more amino acids.

Perfusion The rate of blood flow through tissue, expressed as milliliters of blood flowing per 100 grams of tissue per minute. Poor tumor perfusion can result in tumor hypoxia, and is partially responsible for the resistance of some tumors to chemotherapy or radiotherapy.

Peritoneum The membrane that lines the abdominal cavity and covers most of the organs.

Pharmacokinetics The movement and disposition of drugs within the body; affected by drug uptake, distribution, elimination, and metabolism or transformation to another chemical form.

Pharmacology The study of drugs, including sources, chemistry, actions, and clinical uses.

Plasma cell A cell that secretes large quantities of antibody to a particular antigen; formed when an immature B cell is activated to divide and differentiate into a mature plasma cell.

Pleomorphism Occurring in more than one form, or having many different forms.

Ploidy The number of copies of the genome a cell contains; gametes (egg and sperm) are haploid ($1n$) cells, and contain a single set of chromosomes, while most other cells are diploid ($2n$), and contain two sets of chromosomes; certain tumor cells characteristically are polyploid, and contain a multiple of the haploid number of chromosomes (e.g., $3n$, $4n$), or are aneuploid, and contain an aberrant number of chromosomes that is not a multiple of the haploid number of chromosomes.

Polycythemia An increase above normal in the number of red cells in the blood.

Polyp A general term used for any mass of tissue that projects outward from the surface of an organ or structure; usually benign, but can undergo transformation to a cancerous form.

Polyploid The state of a cell that possesses some multiple of the normal haploid number of chromosomes (e.g., $3n$, $4n$). Certain tumor cells are characteristically polyploid.

Positron emission tomography (PET) Sophisticated medical imaging technique. A radioactively labeled substance (e.g., drug, monoclonal antibody, metabolic substrate, hormone) is administered to the patient and the substance is allowed to localize to some anatomic structure. The patient is then placed within a ring of radioactive decay detectors, which image the decay and roughly localize the labeled structure within the body. PET is very useful for detecting small quantities of bound radioactive substance, but the spatial and temporal resolution is poor, the technique is only useful if a radiolabeled substance can be made to bind to the structure of interest, and PET may require that the patient be given a large dose of radioactivity.

Primary tumor The original tumor present within a body, which can give rise to secondary tumors by metastasis.

Progression Continually increasing severity of disease; the course of a disease in the absence of treatment, or sometimes in spite of treatment; usually accompanied by a worsening of symptoms.

Protein Usually a very large molecule, formed of a linear array of amino acids folded into a characteristic three-dimensional shape. Proteins can serve a multitude of functions, including structural support, movement and contraction, chemical catalysis (e.g., enzymes), immune response (e.g., antibodies), and regulation and integration of bodily functions (e.g.,

hormones). Proteins form about three-fourths of the dry weight of most cells (i.e., 75% of cell weight exclusive of water).

Pyuria The presence of pus in the urine.

Radiation therapy Cancer treatment based on exposure of the tumor to carefully monitored doses of radiation; involves careful treatment planning to minimize radiation toxicity to normal tissues near the tumor.

Radiotherapy See radiation therapy.

Receptor Any molecule capable of selectively binding a particular substance. Receptors are usually proteins, and are often attached to the cell membrane; binding of a particular substance (ligand) to a receptor is often associated with transmission of a regulatory signal into the cell.

Recombinant DNA Altered DNA. DNA resulting from the insertion of a new gene sequence that was not originally present, so that the altered DNA carries tailor-made genetic information.

Remission Decrease in severity of disease symptoms; period of time during which such abatement occurs.

Resection Excision; surgical removal of a segment or part of a tissue.

Resorption Absorption or removal of fluid or tissue. Resorption of normal structures can be associated with the progression of cancer, as in bone resorption caused by multiple myeloma.

Risk factor Any action, exposure, or genetic predisposition that increases the likelihood of contracting disease. The study of risk factors forms a large part of the science of epidemiology.

RNA **R**ibonucleic acid; the molecule that is the repository of genetic information in viruses.

Sarcoma Any of a number of tumors of connective tissue, most of which are highly malignant.

Screening test Any technique used to examine a population of asymptomatic individuals for the presence of occult disease; the ideal screening test is inexpensive, easy, reliable, and accurate.

Secondary tumor Metastatic tumor; tumor formed by metastasis of a primary tumor.

Selection; selection pressure Natural selection; any force or process that tends to act against poorly adapted cells or organisms; the force that drives evolution. In tumors, the scarcity of oxygen or glucose can select against tumor cells that cannot tolerate such scarcity, so selection favors hardy, weedlike cells.

Sensitivity A measure of the ability of a medical or diagnostic test to detect a particular disease or condition; the proportion of individuals with a particular disease who can be identified by a medical test; a test with low sensitivity will fail to identify a large proportion of potential patients.

Specificity A measure of the ability of a medical or diagnostic test to identify a particular disease or condition; the proportion of individuals with a particular disease who can be correctly identified as having that disease (and not some other disease) by a medical test; a test with low specificity will incorrectly identify a large proportion of individuals as potential patients.

Splenomegaly Enlargement of the spleen.

Sporadic Occasional; occurring singly, not grouped.

Squamous cell Scaly cell that forms a layer over an underlying structure.

Stage Tumor classification based on the size of the primary tumor and the extent of dissemination of secondary tumors; a critical indicator of prognosis for the cancer patient. Tumor stage is distinct from tumor grade, which is a classification based on the degree of differentiation of tumor cells.

Stem cell An undifferentiated cell that is capable of differentiating into a range of mature cell types; any morphologically simple cell that can give rise to several different cell types.

Stool Fecal matter; a bowel movement.

Stroma Cells that form a framework of connective tissue common to all organs; opposite of parenchyma, which are cells specific to, or characteristic of, a gland or organ.

Suppressor T cell Cell that acts to suppress immune system function; cell that suppresses the recruitment of new T cells into an immune response, that stops immune cells from attacking tumor cells, or that suppresses production of antibody by B cells. Excessive activity of suppressor T cells is associated with suppression of the immune system, while loss of suppressor function is associated with various autoimmune diseases.

Syndrome An aggregate of signs and symptoms characteristic of a particular disease state.

Systemic Relating to, or affecting, the whole body of the patient.

T cell T lymphocytes are the linchpin of the immune system, serving several different roles and interacting with virtually every other cell of the immune system. An activated or stimulated T cell is able to differentiate

into one of three mature cell types: most (55–65%) immature T cells develop into helper T cells, which provide helper function to other immune cells, while the remaining immature T cells develop into cytotoxic T lymphocytes (CTLs), which directly attack tumor cells, or suppressor T cells, which suppress immune system function.

Telangiectasia Dilation of capillaries or small blood vessels.

Teratogen A drug or other agent that causes abnormal fetal development; the best known teratogen is thalidomide, although alcohol is also a potent fetal teratogen.

Therapeutic ratio A measure of the specificity of a particular treatment for cancer; ratio of tumor cells killed to normal cells killed by therapy. Most current therapies are relatively nonspecific to tumor cells, so these therapies have a low therapeutic ratio.

Thrombophlebitis Inflammation of a vein, with formation of a blood clot at the inflamed site.

Transcription The transfer of genetic information from DNA to RNA; process of synthesizing a molecule of messenger RNA, using DNA as a template.

Transformation Change to a cancerous state; loss of growth regulation of a cell, induced by viral infection, carcinogen exposure, or genetic mutation.

Translation Cellular protein synthesis; the sum of processes by which the genetic information in a molecule of messenger RNA is used to specify the order of amino acids in a particular protein.

Tumor burden The total quantity of tumor cells present in a cancer patient, including both primary and secondary tumors.

Tumorigenesis The creation or production of a tumor; induction of a malignancy.

Tumor-infiltrating lymphocyte (TIL) A tumor-killing lymphocyte; a type of activated T cell that infiltrates into the tumor and selectively kills tumor cells.

Tumor marker A molecule, usually a protein, that is characteristically released by a certain kind of tumor and that can aid in diagnosis of that tumor (e.g., prostate-specific antigen or PSA); tumor markers can be used to screen high-risk individuals for the presence of malignancy, to provide a definitive indicator of malignancy in a patient already identified as a potential cancer patient, to monitor the success of therapy, or to serve as an early indicator of tumor recurrence.

Ultrasound (US) Ultrasonography or echography; a medical imaging technique. Transmitted and reflected sound waves are used to form images that

can be used to locate, measure, and delineate structures within the body. US is able to make real-time images of structures close to the body surface, but image resolution is not good.

Video surgery See laparoscopic surgery.

Viral oncogene A gene, carried by a virus, that is associated with cancer.

Virus An infectious agent of disease; a tiny particle, composed of RNA (or DNA) and protein, which is able to invade cells and cause them to synthesize new viral particles; viruses carry oncogenes that can infect cells and cause malignant transformation and viruses are implicated in causing about 15% of all human cancers.

X ray Roentgen ray; a type of radiation used in radiation therapy; produced by man-made machines and capable of causing ionization of chemicals and destruction of cells.

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